African Journal of Agricultural Research

Volume 11 Number 33 18 August 2016
ISSN 1991-637X

Academic Journals

ABOUT AJAR

The African Journal of Agricultural Research (AJAR) is published weekly (one volume per year) by Academic Journals.

African Journal of Agricultural Research (AJAR) is an open access journal that publishes high-quality solicited and unsolicited articles, in English, in all areas of agriculture including arid soil research and rehabilitation, agricultural genomics, stored products research, tree fruit production, pesticide science, postharvest biology and technology, seed science research, irrigation, agricultural engineering, water resources management, marine sciences, agronomy, animal science, physiology and morphology, aquaculture, crop science, dairy science, entomology, fish and fisheries, forestry, freshwater science, horticulture, poultry science, soil science, systematic biology, veterinary, virology, viticulture, weed biology, agricultural economics and agribusiness. All articles published in AJAR are peer- reviewed.

Contact Us

Editorial Office: ajar@academicjournals.org

Help Desk: helpdesk@academicjournals.org

Website: http://www.academicjournals.org/journal/AJAR

Submit manuscript online http://ms.academicjournals.me/

Editors

Editorial Board

African Journal of Agricultural Research

Table of Contents: Volume 11 Number 33, 18 August, 2016

ARTICLES

African Journal of Agricultural Research

Table of Contents: Volume 11 Number 33, 1 8 August, 2016

ARTICLES

academicJournals

Vol. 11(33), pp. 3065-3073, 18 August, 2016
DOI: 10.5897/AJAR2016.11164
Article Number: B656E4B60029
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Review

Control strategies and breeding efforts in sorghum for resistance to storage weevils

Maletsema Alina, Mofokeng

Agricultural Research Council-Grain Crops Institute, Private Bag X1251, Potchefstroom, 2520, South Africa.

Received 29 April, 2016; Accepted 2 June, 2016

Sorghum (Sorghum bicolor L. Moench) is one of the main staple cereal crops grown worldwide. It is used for food, feed, fodder and bio-ethanol. Biotic and abiotic challenges are the major constraints of the crop. Among the biotic constraints, weevil attack is the most devastating causing yield reduction ranging between 15 and 77%. This paper highlights control strategies and progresses of breeding sorghum towards improved yield and weevil resistance. The use of resistant varieties is an economically feasible, technically easy and environmentally friendly alternative to minimize losses due to storage insect pests. Breeding for post-harvest insect pest resistance is the most important component to improve yield and reduce the impact of weevils. Also to combine resistance to post-harvest insect pests with other desirable plant characters such as high yield, and good quality to provide the basic foundation on which to build an integrated pest management system. Estimation of combining ability to resistance to weevils in sorghum helps in selection of good combiners, and the nature of gene action involved. Marker assisted breeding could have a complementary role in sorghum breeding for introgression of resistance genes and their fast enhancement in succeeding generations in the breeding programmes. This review provides theoretical bases on the progress of breeding sorghum for weevil resistance and control strategies.

Key words: Breeding, insect pests, resistance, sorghum, weevils.

INTRODUCTION

Sorghum, a diploid and a C4 plant belonging to the grass family Gramineae, is one of the most important cereal crops grown in the tropics and sub-tropics of the world. It ranks fifth after maize, rice, wheat and barley (FAO, 2012). It performs well in areas considered marginal for other cereal crops such as maize. It has an ability to withstand harsh conditions including drought and water logging. The sorghum crop is used for food, feed, fodder and bio-ethanol. It is the main source of calories and protein in some regions of Africa and Asia (Waniska and Rooney, 2000). However, its production is hindered by biotic and abiotic constraints. Among the biotic constraints, post-harvest insect pests are the major devastating insect pests attacking the grains during storage. The weevil infestation is encountered on-farm storage where it causes high loss in grain weight in

*Corresponding author. E-mail: MofokengA@arc.agric.za. Tel: +27 72 1222 369.

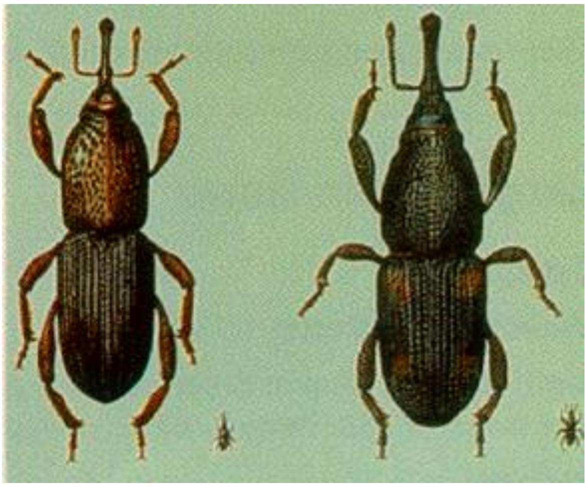

Figure 1. Maize weevil (*S. zeamais*).

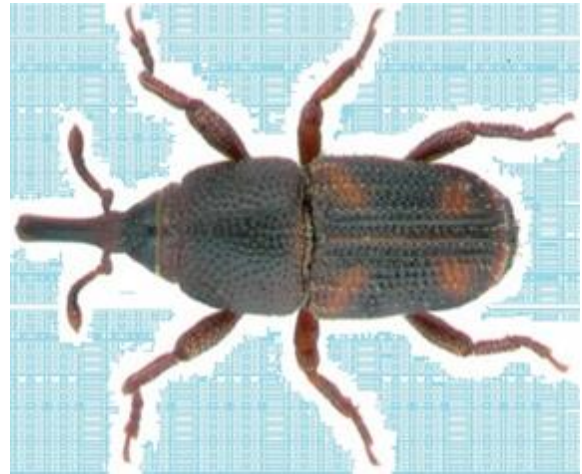

Figure 2. Rice weevil (*S. oryzae*).

addition to deterioration in quality (Giga et al., 1991).

According to Rounet (1992), a huge post-harvest losses and deterioration in quality is one of the major obstacles to achieving food security in developing and under developed countries. Effective control of the weevils would improve the market quality and quantity of sorghum. Various control strategies were employed, and these control strategies include modern and traditional control methods. The methods involved cultural, physical, biological, and chemical controls, as well as host plant resistance. Although the use of chemicals is one of the most common control strategies, it is expensive to smallholder farmers and environmentally undesirable. Host plant resistance is more sustainable and is a major component of integrated pest management which is cheaper and ecologically safer (Abebe et al., 2009; Tefera et al., 2011).

This study seeks to highlight the progresses of breeding sorghum towards improved yield and weevil resistance. Further, the potential and limitations of the conventional and non-conventional breeding methods for sorghum improvement have been reviewed.

Sorghum production constraints

As mentioned earlier, sorghum production and productivity is limited by abiotic and biotic constraints. Abiotic constraints include drought, cold injury and aluminium toxicity among others. Biotic constraints in sorghum include foliar, panicle and grain diseases, weeds, as well as pre- and post-harvest insect pests. The major insect pests of sorghum include stem borers, maize and sorghum aphids, panicle feeding bugs, beetles, bollworms, wireworms, cutworms, shootfly, sorghum midge, armoured cricket, larger grain borer and weevils (Dogget, 1988; Van den Berg and Drinkwater, 1997).

Post-harvest insect pests in sorghum

The post-harvest insect pests that attack sorghum during storage include larger grain borer (*Prostephanus truncates* Horn), rice weevil (*Sitophilus oryzae*) and maize weevil (*Sitophilus zeamais*) (Boxal, 2002; Teetes and Pendleton, 2000). The larger grain borer adults are black or brown and cylindrical in shape with heads facing down. They are larger than 3 to 4 mm and have two strong lateral ridges with sharp edges. The *S. zeamais* is a small snout beetle with adults reaching lengths of between 3 to 3.5 mm and it is capable of flight (Figure 1). The maize weevil has reddish yellow blotches on the wing case. *The S. oryzae* is a small (4 mm) snout reddish brown beetle with faint yellow or red patterns on the wings. It has deep irregular pits behind the head and the snouts can grow up to 1 mm (Figure 2).

The *S. zeamais* and *S. oryzae* occur throughout the warmer, more humid regions of the world, especially where maize is grown (Longstaff, 1981). They have been reported to attack a wide range of crops including wheat, barley, maize, sorghum and other cereal and fruit crops particularly when moisture contents are above 20°C (Anonymous, 2009). The biology of *S. zeamais* and *S. oryzae* has been reviewed in detail by Longstaff (1981). The adults are long-lived. Eggs are laid throughout most of the adult life, although 50% maybe laid in the first 4 to 5 weeks; each female may lay up to 150 eggs. The eggs are laid individually in small cavities chewed into cereal grains by the female; each cavity is sealed, thus protecting the egg, by a waxy secretion produced by the female. The incubation period of eggs are about 6 days at 25°C (Howe, 1952). Eggs are laid at temperatures between 15 and 35°C (with an optimum around 25°C) and at grain moisture contents over 10%. However, rates of oviposition are very low below 20°C or above 32°C, and below 12% moisture content (Birch, 1944). Upon

Figure 3. Weevil damage on sorghum grain.

hatching, the larva begins to feed inside the grain, excavating a tunnel as it develops. The actual length of the life cycle also depends upon the type and quality of grain being infested: for instance, in different varieties of maize, mean development periods of *S. zeamais* at 27°C, and 70% RH have been shown to vary from 31 to 37 days.

Effects of weevils on sorghum

Weevils infest sorghum while still in the field, and deposit eggs in the stored grains and once the larval stage is reached, the larvae feeds on the grains and cause damage (Demissie et al., 2008). The grains are mostly damaged inside and are full of holes (Figure 3). The significant damage happens during grain storage. The damaged grain has reduced nutritional value, low percent germination and reduced weight and market value. Weevil damage leads to quantitative and qualitative deterioration of the grain (Goftishu and Belete, 2014). These losses could be influenced by the storage time and population of insects involved in the infestation. The qualitative loss is attributed to change in biochemical components such as carbohydrates, starch contents and proteins (Danjumma et al., 2009). The commercial value of the infested grain is reduced by contamination with uric acid, insect body fragments, and other toxic substances (Borikar and Tayde, 1979; Gupta et al., 2000). It also predisposes the seeds to attack by storage fungi (Subramanyan et al., 1992). Weevils cause direct damage to the stored grains and also affect their viability and successful planting by smallholder farmers. Globally, yields are affected from a range of 15 to 77% grain losses of insecticide untreated sorghum (Ramputh et al., 1999). Upadhyaya and Ahmad (2011) reported an annual loss of about 10 to 45% annually worldwide.

Strategies for weevil control

The losses of the grain yield can be prevented in various ways including chemical and non-chemical control methods as shown in Table 1 (Hagstrum and Subramanyam, 2006).

Chemical control

The chemical control refers to the application of insecticides before or during storage. Fumigation with chemicals such as Malathion was recommended (Navi et al., 2006). A surface dressing with an insecticide can be applied to prevent insects from entering bins where the grain will be stored. Grain can also be treated with inert dusts, or sprays of synergized pyrethrins. Friction of inert dusts and the weevil's cuticle can cause desiccation and hampers the development of the insect pest (Golob, 1997). The inert dusts, silica gel and aerosol kill insects through physical contact.

Physical control

Sorghum grain can be placed in a low oxygen and CO_2 enriched atmosphere in order to reduce weevil numbers via reduced respiration. Additionally, grain can be placed in a store room with a temperature ranging between 55 to 65°C for 12 h (Upadhyaya and Ahmad, 2011). High temperatures of 55 to 65°C can kill the insect pest's life stages in a life cycle in store houses. Low temperatures also plays a significant role in controlling the insect pests. Temperatures below 12°C makes the weevil inactive and reduces the insect development and kills all immature stages in the life cycle. Keeping grain in store houses with low temperatures provides a long term seed storage and high mortality of insect pests.

Cultural control

The cultural control method involves keeping the store warehouses clean at all times, for example removal of cracked seeds, egg shells and dead larvae. Store houses should be kept clean between the harvests and infested residues must be removed and burned. Further, the crop can be harvested early, immediately after maturity. Selections of only un-infested material should be made for grain storage. The clean grain should be stored in containers that are fitted with insect proof glass, or refrigeration or deep freeze. In addition, the storage should be ventilated to get rid of any moisture as the insect pests reproduce rapidly in moist areas. The moisture of the seed should be 12% or less during storage. This will help to reduce insect pest numbers and their survival and development will be hindered.

Biological control

The biological control includes the use of parasitoids

Table 1. Strategies used to control storage weevil infestation.

Techniques/strategies	References
Malathion application	Navi et al. (2006)
Inert dusts	Golob (1997)
Bacillus thuringiensis	Abdel-Razek (2002)
Growth regulators: Methropene and hydropene	Mian and Mulla (1982)
Host resistance	Borikar and Tayde (1979), Shazali (1982), Fademula and Horber (1984), Wongo and Pedersen (1990)
Low aeration	Maier et al. (2002), Reed and Authur (1998)
Wood ash	De Groot (2004)
Extracts	Karthikeyan et al. (2009)
Plant parts	Karthikeyan et al. (2009)
Plant powders	Suleiman et al. (2012), Danjumma et al. (2009), Mukherjee and Joseph (2000)
Natural oils	Koul et al. (2008), Mollalei et al. (2011)
Volatile substances: Methyl salicylate	Jayasekara et al. (2005), Liu et al. (2006)

which must be introduced early in the storage facility to outnumber the insect pests. These parasitoids include species such as *Anisopteromalus calandrae, Cephalomia tarsalis, Lanophagus distinguendus and Theocola xelegans* to feed on the insect pests. *Beauveria bassiana* and *Bacillus thuringiensis* can also be used as biological insecticides for control of maize weevil. The *B. thuringiensis* produces Bt toxins that are harmful to the insect pests. Sometimes *B. thuringiensis* is mixed with some botanicals such as plant essential oils and their chemical constituents and results in killing of insect pests in higher rates. The essential oils of many plant species are known to have repellent and insecticidal activities (Koul et al., 2008; Mollaei et al., 2011). *Bacillus thuringiensis* strains are also used to control Coleopteran pests of stored wheat (Abdel-Razek, 2002). Moreover, insect growth regulators such as methropene and hydropene can be used to reduce insect pest populations of *S. oryzae* (Mian and Mulla, 1982).

Host plant resistance

The use of varieties resistant to storage weevils is another strategy for preventing yield losses due to storage weevils. In areas where storage facilities are inadequate, stored grain resistance might be used alone or as an adjunct to chemical control to protect yields. Pramod et al. (2002) reported presence of resistance to rice weevil (*S. oryzae*) when using A/B lines, R lines, commercial varieties, germplasms, mutants and locals. The study showed a need to increase the levels of resistance among parental lines and hybrids to ensure a better protection from rice weevil infestation in stored sorghum. Resistance to *S. oryzae* was also reported in sorghum by Prasad et al. (2015) and Kudachi and Balikai (2014). Various researchers studied and reported resistance of sorghum to storage weevils (Mannechoti, 1974; White, 1975, Doraiswamy et al., 1976; Borikar and Tayde, 1979; Shazali, 1982; Fademula and Horber, 1984; Wongo and Pedersen, 1990). Some sorghum genotypes were found to possess variable degrees of resistance viz. progeny emergence (Borikar and Tayde, 1979), low larval penetration (Shazali, 1982; Wongo and Persen, 1990), increased progeny emergence (Adentuji, 1988) and low loss in seed weight (M'bata, 1992). Combining early planting with early harvest with resistant varieties proved to be effective for management of maize weevils in the stores in Niger Delta-agro-ecological zone (Zakka et al., 2015).

Integrated pest management (IPM)

Integrated pest management strategies can manage to control insect pests in bulk grain (Hagstrum et al., 1999). Once the grain is stored, the biophysical conditions in the bin can be monitored and modified for insect control by:

1. Removing grain samples and counting the number of live and/or dead insects
2. Using insect traps to estimate insect population
3. Using automatic insect traps to detect insect activity at different spots in the grain mass
4. Using temperature sensors and data loggers to collect hourly temperature measurements at different spots in the grain mass.

Based on the monitoring outcome, one or a combination of control strategies can be implemented appropriately. For instance, using low aeration to control the biophysical conditions in the storage bin to control insect population growth rate (Maier et al., 2002; Reed and Arthur, 1998). The grain can be kept at lower temperatures, for example

Figure 4. Farmer's traditional granary for grain storage.

16°C which is the lowest temperature limit for survival, development and reproduction of the storage pests. Development and use of improved grain cultivars with resistance to storage pests and pre-harvest pests will provide a key elementary control in IPM.

Farmer's traditional control strategies of weevils

Traditionally farmers in rural areas in some parts of India and Africa use traditional granaries to store their grains (Figure 4). The granaries are found not to be very effective against storage pests. Hence, a need for suitable storage facilities and management technologies following the cultural control methods. This lack somehow forces farmers to sell their grain immediately after harvest. As a result, farmers receive low market prices for any surplus grain they may produce due to storage weevil damage (Kimenju et al., 2009).

Physical control

Traditionally, farmers apply wood ash (*Acacia* and *Casuariana* spp.) (De Groot, 2004), or clay to stored grain in order to cause insects to die from desiccation. Farmers believed that ash prevent grain losses up to an extent of 80% (Karthikeyan et al., 2009). Farmers using the ash strategy stored sorghum grains for 6 months without any storage pests' problems. Farmers further use sands and soil components on seed grain to control storage pests. The grain will be covered by sand particles which forms a protective layer of the stored grain (Golab and Webley, 1980). For instance, treating *Vigna radiata* with inert clay before storage lead to 100% mortality of *Callosobruchus chinensis* within 24 h. The inert clay coat can control insect pests up to twelve months under ambient conditions (Babu et al., 1989). Smallholder farmers store their grains in bags and put them in holes dug inside the cattle kraal and cover with the cow dung. This strategy seemed to be effective against storage pests as the ammonia from the faeces repelled the weevils. According to Karthikeyan et al. (2009) farmers preferred jute gunny bags for short term storage of grain to be used as seed materials for future sowing. The gunny bags were treated with neem seed kernel extract. The practice involved preparation of neem seed kernel extract (NSKE) and then treating the gunny bags with the extract before storage by soaking them in the solution (Karthikeyan et al., 2009). In addition, fresh pungam (*Pongamia glabra*) leaves were placed in layers between the gunny bags arranged one above the other in the storerooms. The strong odour from the leaves acts as a repellent against the weevils. Farmers also use paddy husks to store grain. For instance, the paddy grains (*Oryza sativa*) were stored in earthen pods and placed paddy husk in top layer (5 cm) above it. The storage pests did not prefer the grain stored with paddy husk. Lastly, farmers made traditional mud pots of different capacity and sizes with the clay soil. Firstly the grain were sun dried and cleaned before storing in pots. Farmers placed a circular ring-like structure made with paddy straws on the floor. Above the ring, mud pots filled with grain are placed and then arranged one on top of the other and the top most pots were closed with the lead. This is usually in the house at the corner. The grains were kept for about six months, and then were sun dried and again re-stored in the mud pots for control of storage insect pests.

Use of plant powders

Non-chemical control of weevils refers to the use of physical, cultural and biological control methods. Danjumma et al. (2009) used plant powders of *Nicotiana tabacum, Allilumsativum, and Zingiber officinale* to control maize weevils. Suleiman et al. (2012) reported powders of *Jatropha curcas, Euphorbia balsamifera*L*., Lawsonia ineris*L. and *Leptadenia hastate* L. collected from the bushes to control weevil infestation on sorghum grains. The Japanese mint (*Mentha arvensis*) oil was found effective as fumigant against *Sitophilus oryzae* in sorghum (Mukherjee and Joseph, 2000). *Inula graveolens* and soap berry are mixed with grain to protect grains against weevils (De Groot, 2004). Sweet flag (*Acorus calamus*) is one of the indigenous methods used as a plant powder mixed with grain and the grain can be protected for up to six months. This strategy was used for more than 40 years to store seeds of pulses, cereals and oil crops. In the study by Bhanderi et al. (2015) sweet flag powder, custard apple seed powder and neem seed kernel powder were the most effective powders in controlling populations of weevils in sorghum.

Natural oils

The essential oils of many plant species have repellent and insecticidal activities (Koul et al., 2008; Mollalei et al., 2011). Natural products show little detrimental effects on the environment and non-target organisms and is continuously evaluated for their pesticidal effects (Matthews, 1993). These oils have shown an effect on the oviposition and growth inhibitory activity (Triphati et al., 2001).

Volatile substances

In addition, volatile constituents, such as methyl salicylate from *Securidacalonga pedunculata* exhibited repellent and toxic properties against *S. zeamais* and *Rhizopertha dominica* (Jayasekara et al., 2005). The active component from leaves of *Artimisia princepi* and seeds of *Cinnamomum camphora* (L.) have shown repellent and insecticidal activity against *S. oryzae* and *Bruchus rugimanus* (Liu et al., 2006). The volatile constituent, di-n-propyl disulphide extracted from the seed of neem, *Azadirachta indica*, is toxic when applied as a fumigant to *Tribolium castaneum* adults and larvae of *S. oryzae* adults. These plant products show toxicity against pests of stored grain and furthermore, provide prolonged protection to seeds that may be due to high mortality of adult insect besides the effect on oviposition and low hatching (Huang and Subramanyam, 2004).

Conventional breeding for weevil resistance in sorghum

There is little progress in developing insect-resistant high yielding sorghum varieties for cultivation by the farmers. This is largely because of the lack of knowledge on inheritance of the agronomic and morphological characteristics associated with insect resistance and grain yield (Sharma et al., 2005; Riyazaddin et al., 2015). Traditional breeding methods such as germplasm evaluation and enhancement, backcrossing, pedigree selection, and recurrent selection continue to play an important role in developing insect-resistant cultivars with major resistance genes (Huang et al., 2013). In such breeding approaches, sorghum breeders search for genetic variability for insect resistance and then incorporate the desired gene into breeding lines, leading to the development of resistant commercial cultivars or hybrids.

Since sorghum is a self-pollinated crop, most breeding methodologies are based on the production of segregating populations followed by selection in segregating population. The selections are usually allowed to self-pollinate during selection to produce homozygous uniform lines (pure lines). In hybrid breeding programmes, these lines will be test crossed to measure their value as parental lines. Although the aforementioned breeding methods are employed in breeding for insect pest resistance pest resistance, there is still limited breeding studies conducted on resistance to weevil damage in sorghum *per se*.

Other studies on breeding for resistance to weevils using traditional breeding methods have been reported on maize but limited on sorghum. Kasozi et al. (2015) used two cycles of modified S1 recurrent selection to study resistance to maize weevil in maize. The authors found that the modified S1 recurrent selection was effecttive in improving Longe5 for maize weevil resistance.

Marker assisted breeding

Genetic resistance in sorghum, where possible, should be combined with other desirable plant characters such as high yield and good quality grain providing a basic foundation on which to build integrated pest management systems. A promising strategy should be based on gene pyramiding and development of cultivars with multiple resistances to insect pests. Many studies were conducted in identifying the quantitative trait loci (QTL) regions of different traits associated with insect resistance as well as the morphological and agronomic traits (Satish et al., 2009; Srinivas et al., 2009; Aruna et al., 2011; Nagaraja Reddy et al., 2013, 2014).

Based on the present inheritance studies of the agronomic and morphological traits and as well as the QTL information available, one can effectively plan suitable breeding strategies for sorghum improvement. In contrast to the conventional approaches that takes about six to eight generation to transfer a trait within a species into high yielding, locally adapted cultivars, and the non-conventional breeding approaches such as marker-assisted selection (MAS) allows rapid introgression of the resistance genes and ultimately gene pyramiding into the high-yielding varieties and hybrids. It further allows early selection of breeding materials and cloning of the important resistance genes for sorghum improvement via map-cloning method. Use of DNA markers for indirect selection offers great potential gains for quantitative traits with low heritability, as these are the most difficult traits to work with in the field using direct phenotypic selection. However, little work has been done on the use of molecular markers on weevil resistance gene mapping and transfer to the locally adapted high yielding sorghum varieties.

Nonetheless, Silverio et al. (2009) mapped the QTLs associated with resistance to maize weevil in maize using 151 RFLP markers, and further found that the genetic effects were mainly dominant for grain damage, grain weight losses and maize weevil susceptibility index, and additive for number of adult progeny. Castro-Alvarez et al. (2015) identified a total of 15 QTLs for maize weevil resistance parameters located on six chromosomes in a RIL maize population using SSR markers.

The genetics of weevil resistance in sorghum

Information on inheritance of agronomic and morphological traits is useful for improving genotypic performance across environments. An understanding of the inheritance of morphological and agronomic traits will be helpful in combining the genes for insect resistance and desirable agronomic traits and grain characteristics to increase production and productivity of sorghum. The genetic effect can either be additive, dominant or epistatic and in rare case over dominance. According to Griffing (1956), general combining ability (GCA) and specific combining ability (SCA) are used to estimate gene effects. The GCA is used to estimate additive genetic effects while SCA estimates the non-additive components.

Mohammed et al. (2015) studied the genetic analysis of agronomic and morphological traits in sorghum in relation to insect resistance. The authors found that the GCA/SCA, and the predictability ratios indicated predominance of additive gene effects for majority of the traits studied. The significance of reciprocal combining ability effects for days to 50% flowering, plant height and 100 seed weight, suggested maternal effects for inheritance of these traits. Plant height and grain yield across seasons, days to 50% flowering, inflorescence exsertion, and panicle shape in the post-rainy season showed greater specific combining ability variance, indicating the predominance of non-additive type of gene action/epistatic interactions in controlling the expression of these traits.

Additive gene action in the rainy season, and dominance in the post-rainy season for days to 50% flowering and plant height suggested genotype by environment interactions for these traits. Other authors reported high GCA/SCA and predictability ratios for 100 seed weight in the post-rainy season indicated the predominance of additive gene action, whereas both additive and non-additive gene action was observed in the rainy season. Grain yield exhibited higher SCA variance suggesting the predominance of dominance (non-additive) type of gene action (Hovny et al., 2000; Umakanth et al., 2002; Girma et al., 2010).

However, the importance of both additive and non-additive gene action was observed for 100 seed weight by Toure et al. (1996). The combining ability analysis is useful for understanding of the nature of gene action, and has been used by breeders for selection of suitable parents for the crossing programmes.

Effects of genotype by environment interaction on weevil resistance in sorghum

Studies on the effects of genotype by environment interaction on the resistance of maize and rice weevils in sorghum are not yet documented. Sorghum improvement efforts in the development of cultivars and hybrids with high grain yield under diverse environmental conditions are needed. Osiru et al. (2009) suggested that knowledge of genotype performance in different agro-ecologies is critical in cultivar development. Selecting genotypes that interact less with the environments in which they are grown would be beneficial. Maize hybrids were tested in nine environments in Kenya and Ethiopia for resistance to weevils, larger grain borer and other foliar diseases (Tefera et al., 2013).

The results indicated that the performances of the hybrids were not consistent across environments for all traits tested as evident from the significant genotype by environment interactions and two hybrids were showed resistance to larger grain borer and maize weevil (Tefera et al., 2013). A study involving inbred lines and ten top best crosses was reported in maize for combining ability gene action and resistance to weevils in maize (Gafishi et al. 2010). The inbred lines and the crosses were tested in two environments where the across site analysis of genotype by environment for yield revealed that the testcrosses were environment specific. It is possible to identify genetic variability within an environment although stable performance is required.

CONCLUSION AND RECOMMENDATIONS

Postharvest insect pests such as maize and rice weevils are the major limiting factor in sustainable production of sorghum. Host plant resistance is one of the more effective control strategies of postharvest insects without the undesirable effects of pesticides. Continuous research efforts on the identification of new sources of resistance to these pests and breeding should be the major objectives of breeding programmes. A promising strategy for sorghum improvement should be based on gene pyramiding and development of cultivars with multiple resistance to insect pests. Also to combine resistance to post-harvest insect pests with other desirable plant characters such as high yield, and grain quality to provide the basic foundation on which to build integrated pest management system. There is an urgent need for transfer of various insect resistance genes into cytoplasmic male sterile, maintainer, and restore lines so as to develop hybrids with increased levels and diverse mechanisms of resistance to target pests. Furthermore, there is a need for genotypes that show good combining ability effects for weevil resistance, yield and other agronomic characters, as well as selection of genotypes that interact less with the environment in which they are grown.

Conflict of Interests

The authors have not declared any conflict of interests.

REFERENCES

Abdel-Razek AS (2002). Comparative study on the effect of two *Bacillus thuringiensis* strains of the same serotype on three Coleopteran pests of stored wheat. J. Egypt Soc. Parasitol. 84.32(2):415-424.

Abebe F, Tefara T, Mugo S, Beyene Y, Vidal S (2009). Resistance of maize varieties to the maize weevil *Sitophilus zeamais* (Motsch.) (Coleoptera: Curculionidae). Afr. J. Biotechnol. 8:5937-5943.

Adentuji JF (1988). A study of the resistance of some sorghum seed cultivars to *Sitophilus oryzae* (l.) Coleptera: Curculionidae). J. Stored Prod. Res. 24:67-71.

Anonymous (2009). Maize weevil *Sitophilus zeamais* (Motsch.) Canadian Grain Commission.

Aruna C, Bhagwat V, Madhusudhana R, Sharma V, Hussain T, Ghorade RB, Khandalkar HG, Audilakshmi S, Seetharama N (2011). Identification and validation of genomic regions that affect shootfly resistance in sorghum [*Sorghum bicolor* (L.) Moench]. Theor. Appl. Genet. 122:1617-1630.

Babu TR, Reddy VS, Hussaini SH (1989). Effect of edible and non-edible oils on the development of the pulse beetle (*Callosobruchus chinensis* L.) and on viability and yield of mung bean (*Vignaradiata* [L.]Wilczek). Trop. Sci. 29:215-220.

Bhanderi GR, Radadia GG, Patel DR (2015). Ecofriendly management of rice weevil *Sitophilus oryzae* (Linnaeus) in sorghum. Indian J. Entomol. 77(3):210-213.

Birch LC (1944). Two strains of *Calandra oryzae* L. (Coleoptera). Aust. J. Exp. Biol. Med. Sci. 22:271-275.

Borikar PS, Tayde DS (1979). Resistance in sorghum to *Sitophilus oryzae* Linn. Proc. Indian Acad. Sci. 88B:273-276.

Boxal RA (2002). Damage and loss caused by the larger grain borer *Prostephanus truncates*. Int. Pest Manage. Rev. 7:105-121.

Castro-Alvarez FF, William M, Bergvinson DJ, Garcia-Lara S (2015). Genetic mapping of QTL for maize weevil resistance in a RIL population of tropical maize. Theor. Appl. Genet. 128:411-419.

Danjumma BJ, Majeed Q, Manga SB, Yabaya A, Dike MC, Bamaiyi L (2009). Effect of some plants powders in the control of *Sitophilus zeamais* Motsch (Coleoptera: Curculionidae) infestation on maize grains. Am. Eur. J. Sci. Res. 4(4):313-316.

De Groot I (2004). Protection of stored grains and pulses. Agrodok 18.Agromisa Foundation. Wageningen.

Demissie G, Tefera T, Tadesse A (2008). Importance of husk covering on field infestation of maize by *Sitophilus zeamais* Motsch. (Coleoptera: Curculionidea) at Bako, Western Ethiopia. Afr. J. Biotechnol. 7:3774-3779.

Dogget HD (1988). Sorghum.2nd ed. Longman Group UK, Green and Co. Ltd, London, Harlow, England.

Doraiswamy V, Subramanian TR, Dakshinamurthy A (1976). Varietal preference in sorghum for the weevil, *Sitophilus oryzae* L. Bull. Grain Technol. 14:107-110.

Fademula A, Horber E (1984). Resistance of sorghum varieties to *Sitophilus oryzae* and *Sitotroga carealella*. In: Proc 3rd Intl. Working Conf. Stored-Product Entomology 23-28 October. Kansas State University, Manhattan, Kansas, USA. pp. 418-434.

Gafishi MK, Tumutegyereize JK, Asea G (2010). Inheritance of resistance to maize weevil *Sitophilus zeamais* (Motschulsky) in maize inbred lines and determination of their heterotic grouping. Second RUFORUM Biennial Meeting 20-24 September, Entebbe, Uganda.

Giga DP, Mutumeerwa S, Moya G, Neeley D (1991). Assessment and control of losses caused by insect pests in small farmer's stores in Zimbabwe. Crop Prot. 10:287-292.

Girma M, Amsalu A, Ketema B (2010). Combining ability for yield and its components in Ethiopian sorghum [*Sorghum bicolor* (L.) Moench] landraces. East Afr. J. Sci. 4:34-40.

Goftishu M, Belete K (2014). Susceptibility of sorghum varieties to the maize weevil *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae). Afr. J. Agric. Res. 9(31):2419-2426.

Golab P, Webley DJ (1980). The use of plants and minerals as protectants of stored product. Tropical product Institute G138.Post-harvest Pest and Quality Section Natural Resource Institute. Cathan, U.K. p. 32.

Golob P (1997). Current status and future perspectives for inert dusts for control of stored product insects. J. Econ. Entomol. 33:69-79.

Griffing B (1956). Concept of general and specific combining ability in relation to diallel crossing system. Aust. J. Biol. Sci 9:463-493.

Gupta AK, Bahal SR, Awashi BK, Verma RA (2000). Reaction protein, starch and ash constituent of different varieties of maize on growth and development of *Sitophilus oryzae* L. Indian J. Entomol. 62:375-381.

Hagstrum DW, Subramanyam BH (2006). Fundamentals of stored product. Entomology, American Association of Cereal Chemists (AACC). St. Paul, Minnesota.

Hagstrum DWC, Reed C, Kenkel P (1999). Management of stored wheat insect pests in the USA. Int. Pest Manag. Rev. 4:127-142.

Hovny MRA, El-Naggouly OO, Hassaballa EA (2000). Combining ability and heterosis in grain sorghum [*Sorghum bicolor* (L.)Moench]. Assiut J. Agric. Sci. 31:1-16.

Howe RW (1952). The biology of the rice weevil, *Calandraoryzae* (L.). Ann. Appl. Biol. 39(1):68-180.

Huang F, Subramanyam B (2004). Responses of *Corcyra cephalonica* (Stainton) to primiphos-methyl, spinosad and combinations of pirimiphosmthyl and synergized pyrethrins. Pest Manag. Sci. 60(2):191-198.

Huang Y, Sharma HC, Dhillon MK (2013). Bridging conventional and molecular genetics of sorghum insect resistance. *In*: Paterson A.H. (Ed.) Genomics of the saccharine, Plant Genetics and Genomics: Crops and Models. Springer Science+ Business Media, New York. pp. 367-389.

Jayasekara TK, Stevenson PC, Hall DR, Belmain SR (2005). Effect of volatile constituents from *Securidaca long pedunculata* on insect pests of stored grain. J. Chem. Ecol. 31(2):303-313.

Karthikeyan C, Veeraravathatham D, Karpagam D, Ayisha Firdouse S (2009). Traditional storage practices. Indian J. Trad. Knowl. 8(4):564-568.

Kasozi LC, Derera J, Tongoona P (2015). Response of maize population "Longe5" to two cycles of modified S_1 recurrent selection for resistance to maize weevil. Euphytica 204(3):587-598.

Kimenju SC, De Groote H, Hellin H (2009). Preliminary Economic Analysis: Cost effectiveness of the use of improved storage methods by small scale farmers in east and southern Africa countries. International Maize and Wheat Improvement Centre (CIMMYT). P. 17.

Koul O, Walia S, Dhaliwal GS (2008). Essential oils as green pesticides: Potential and Constraints. Biopestic. Int. 4(1):63-84.

Kudachi DC, Balikai RA (2014). Evaluation of *rabi* sorghum genotypes against rice weevil, *Sitophilusoryzae* in storage. Indian J. Entomol. 76(2):87-91.

Liu CH, Mishar AK, Tan RX, Yang H, Shen YF (2006). Repellent and insecticidal activities of essential oils from *Artemisia princeps* and *Cinnamomum camphora* and their effect on seed germination of wheat and broad bean. Technol. Bioresour. 97(15):1969-1973.

Longstaff BC (1981). Biology of the grain pest species of the genus *Sitophilus* (Coleoptera: Curculionidae): A critical review. Protect. Ecol. 2:83-130.

M'bata GN (1992). Egg distribution on seeds by *Calllosobruchus subinnotatus* (Pic.) (Coleoptera: Bruchidae). J. Stored Prod. Res. 28:301-139.

Maier DE, Ileleji KE, Woloshuk CP, Szabela DA (2002). The effect of temperature management on *Sitophilus zeamais,Tribolium castaneum* and *Plodia interpunetella* in maize: Summer 2001 Pilot Bin Trials. *In*: Careland, P.F., D.M. Armitage, C.H. Bell, P.M. Cogan and E. Highley (eds) Advances in stored product protection. Proceedings of 8th International Working Conference on Stored Product Protection. York, U.K. CAB International, Willingford, UK.

Mannechoti P (1974). Studies of resistance of 92 sorghum and 38 maize cultivars to four species of stored product insects. MSc thesis. Kansas State University, Manhattan, Kansas.

Mian LS, Mulla MS (1982). Biological and environmental dynamics of insect growth regulators (IGRs) environmental and health impacts of juvenile hormone analogue, methopreneas used against Diptera of public health importance. In: Gunther, F.A. (ed.) In Residue Reviews. pp. 27-112.

Mollaei M, Izadi H, Hossein D, Majid A, Reza RK (2011). Bioactivity of essential oil from *Satureja hortensis* (Laminaceae) against three stored product insect species. Afr. J. Biotechnol. 10(34):6620-6627.

Mukherjee SN, Joseph M (2000). Medicinal plant extracts influencing insect growth and reproduction. J. Med. Arom. Plant Sci. 22(1):38.

Nagaraja RR, Madhusudhana R, Murali Mohan S, Chakravarthi DVN, Mehtre SP, Seetharama N, Patil JV (2013). Mapping QTL for grain yield and other agronomic traits in post-rainy sorghum [*Sorghum*

bicolor (L.) Moench]. Theor. Appl. Genet. 126:1921-1939.
Nagaraja RR, Madhusudhana R, Murali Mohan S, Seetharama N, Jagannatha Vishnu P (2014). Detection and validation of stay-green QTL in post-rainy sorghum involving widely adapted cultivar, M35-1 and a popular stay-green genotype B35. BMC Genomics 15:909.
Navi SS, Deshpande VK, Kulloli SD (2006). Influence of seed colouring on rice weevil infestation during storability of hybrid sorghum. Karnataka J. Agric. Sci. 19(2):287-290.
Osiru MO, Olanya OM, Adipala E, Kapinga R, Lemaga B (2009). Yield stability analysis of *Ipomoea batatas* L. cultivars in diverse environments. Aust. J. Crop Sci. 3(4):213-220.
Pramod KKR, Singh BU, Dharma R (2002). Sorghum resistance to the rice weevil, *Sitophilus oryzae* (L.): Antixenosis. Insect Sci. Appl. 22(1):9-19.
Prasad GS, Babu KS, Sreedhar M, Padmaja PG, Subbarayudu B, Kalaisekar A, Patil JV (2015). Resistance in sorghum to *Sitophilus oryzae* (L.) and its association with grain parameters. Phytoparasitica 43:391-399.
Ramputh A, Techome A, Bergvinson DJ, Nozzolillo C, Arnason JT (1999). Soluble phenolic content as an indicator of sorghum grain resistance to *Sitophilus oryzae* (Coleptera: Curculionidae). J. Stored Prod. Res. 35:57-64.
Reed C, Arthur F (1998). Conditioning practices and their effects on quality of corn stored on Kansas Farms. Appl. Eng. Agric. 14:623-630.
Riyazaddin MD, KaviKishor PB, Ashok Kumar A, Belum Reddy VS, Rajendra SM, Sharma HC (2015). Mechanisms and diversity of resistance to sorghum shoot fly, *Atherigona soccata*. Plant Breed. 134:423-436.
Rounet G (1992). Maize. The tropical agriculturist. CTA, Macmillan, London.
Satish K, Srinivas G, Madhusudhana R, Padmaja PG, Nagaraja Reddy R, Murali Mohan S, Seetharama N (2009). Identification of quantitative trait loci for resistance to shoot fly in sorghum [*Sorghum bicolor* (L.) Moench]. Theor. Appl. Genet. 119:1425-1439.
Sharma HC, Reddy BVS, Dhillon MK, Venkateswaran K, Singh BU, Pampapathy G, Folkerstma RT, Hash CT, Sharma KK (2005). Host plant resistance to insects in sorghum: present status and need for future research. Int. Sorghum Millets Newslett. 46:36-43.
Shazali MEH (1982). The biology and population ecology of four insect pests of stored sorghum with particular reference to competition and succession. PhD thesis, University of Reading, Reading, UK.
Silverio G, Mirelle MK, Mateo V, David JB (2009). Mapping of QTL associated with maize weevil resistance in tropical maize. Crop Sci. 49(1):139-149.
Srinivas G, Satish K, Madhusudhana R, Nagaraja Reddy R, Murali Mohan S, Seeharama N (2009). Identification of quantitative trait loci for agronomically important traits and their association with genic-microsatellite markers in sorghum. Theor. Appl. Genet. 118:1439-1454.
Subramanyay B, Wright VF, Fleming EE (1992). Laboratory evaluation of food habits for their relative ability to retain three species of stored product beetles. J. Agric. Entomol. 9:117-127.
Suleiman M, Ibrahim ND, Majeed Q (2012). Control of *Sitophilus zeamis* (Motsch) [Coleoptera: Curculionadae] on sorghum using some plant powders. Int. J. Agric. For. 2(1):53-57.
Teetes GL, Pendleton BB (2000). Insect pests of sorghum.*In*: Smith, CW and Frederikson, RA (eds.), Sorghum: Origin, history, technology, and production. Wiley Series in Crop Science, New York. . pp. 443-495.
Tefera T, Mugo S, Beyene Y, Karaya H, Gakunga J, Demissie G (2013). Postharvest insect pest and foliar disease resistance and agronomic performance of new maize hybrids in East Africa. Int. J. Plant Breed. Genet. 7(2):92-104.
Tefera T, Mugo S, Likhayo P (2011). Effects of insect population density and storage time on grain damage and weight loss in maize due to the maize weevil *Sitophilus zeamais* and larger grain borer *Protephanus truncates*. J. Agric. Res. 6:2249-2254.
Toure A, Miller FR, Rosenow DT (1996). Heterosis and combining ability for grain yield and yield components in guinea sorghums. J. Afr. Crop Sci. 4:383-391.
Triphati AK, Prajapati V, Aggarwa KK, Kumar S (2001). Insecticidal ovicidal activity of the essential oil of *Anethum sowa* Kurz against *Callosobruchus maculatus* F. (Coleoptera:Bruchidae). Insect Sci. Appl. 31(1):61-66.
Umakanth AV, Madhusudhana K, MadhaviLatha P, Hema K, Kaul S (2002). Genetic architecture of yield and its contributing characters in post rainy season sorghum. Int. Sorghum Millets Newslett. 43:37-40.
Upadhyay RK, Ahmad S (2011). Management strategies for control of stored grain insect pests in farmer stores and public ware houses. World J. Agric. Sci. 7(5):527-549.
Van den Berg J, Drinkwater TW (1997). Field guide to identification of sorghum pests in Southern Africa. Agricultural Research Council-Grain Crops Institute, Potchefstroom, South Africa.
Waniska RD, Rooney LW (2000). Structure and chemistry of the sorghum caryopsis. In: Smith, C.W., and R.A. Frederiksen (Eds.), Sorghum: origin, history, technology, and production. Wiley, New York, USA. pp. 649-688.
White SC (1975). Laboratory studies on levels and causes of insect resistance in stored sorghum varieties. MSc Thesis. Kansas State University, Manhattan, Kansas, USA.
Wongo LE, Pedersen JR (1990). Effect of threshing different sorghum cultivars on *Sitotroga cerealella* (Oliv.) and *Sitophilus oryzae* (L.) (Lepidoptera: Gelechiidae and Coleptera: Curculionidae). J. Stored Prod. Res. 26(2):89-96.
Zakka U, Lale NES, Umeozor OC (2015). Efficacy of combining varietal resistance with harvest time and planting date for the management of *Sitophilus zeamais* Motschulsky infestation in stored maize. J. Stored Prod. Res. 60:31-35.

academicJournals

Vol. 11(33), pp. 3074-3081, 18 August, 2016
DOI: 10.5897/AJAR2014.9108
Article Number: 5523E5660031
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Acceptability of cotton fabric treated with dye extracted from Roselle (*Hibiscus sabdariffa*) calyces based on its phytochemical composition and evaluation of organoleptic attributes

Ozougwu, Stella U.[1]* and Anyakoha, Elizabeth U.[2]

[1]Department of Home Science, Nutrition and Dietetics, Faculty of Agriculture, University of Nigeria, Nsukka, Enugu State, Nigeria.
[2]Department of Vocational Teacher Education Faculty of Education, University of Nigeria, Nsukka Enugu State, Nigeria.

Received 1 September, 2014; Accepted 23 April, 2016

The study evaluated the acceptability of 100% cotton fabric treated with dye extracted from roselle (*Hibiscus sabdariffa)* calyces based on its phytochemical compositions and organoleptic attributes. A quasi-experimental design was adopted by the study and was carried out at the University of Nigeria Nsukka, Enugu State, Nigeria. The study population comprised of 41 panelists made up of 17 Lecturers and 24 Postgraduate students drawn from the university. Spectrophotometric and gravimetric methods were adopted for the quantitative analysis of phytochemical constituents of extracts from roselle calyces. Questionnaire was used to collect data on the organoleptic attributes and acceptability of cotton fabric treated with roselle dye and data were analyzed using descriptive statistics including percentages, means, and standard deviation. A null hypothesis was tested at 0.05 level of significance using t-test. The following phytochemical components were present in roselle dye extract: carotenoid (1.96%), flavonoid (0.02%), lutein (0.03%), polyphenol (0.12%), tannin (0.88%) per 100 g of roselle calyces. Organoleptic attributes of cotton fabric treated with dye extracted from roselle calyces identified include: Fairly warm maroon colour hue, fairly light value, fairly brilliant chroma, smooth and fairly soft textures, odourless and even shade colour which were all accepted as good attributes of dye on fabrics by both categories of evaluators. There were no significant differences (P>0.05) in the mean rating responses of both categories of evaluators on the acceptability of the organoleptic attributes of cotton fabric treated with roselle dye. The null hypothesis was accepted at 0.05 level of significance. Roselle dye has good organoleptic attributes and could be used for 100% cotton fabric colouration.

Key words: Phytochemical, organoleptic attributes, roselle dye, cotton fabric.

INTRODUCTION

Roselle calyces (*Hibiscus sabdariffa*) plant has been extensively utilized for various purposes for making beverages, manufacture of newsprint and found useful in medicinal and pharmacological fields and for making food colourants (Schippers, 2000; The Technical Centre for Agricultural and Rural Co-operation ACP-EU, 2006) but has not been adequately explored for its dye for fabric colouration despite its large pigment content. Dye is an organic chemical compound which imparts permanent colour to other materials. The scarcity of quality dyes in sustainable supplies and the challenging and threatening effects in the nation's textiles and clothing, wood, food, paper, photography, leather and leather product industries as well as in educational institutions and at homes calls for urgent need for research and development efforts into sourcing and exploitation of locally available plant dyes in sustainable supplies to substitute and/or supplement the imported ones (Onwualu, 2006). A quality dye should among other factors, be soluble in water or dispersible in a solvent resulting in evenness of shade or level dyeing in fabric colouration, have pleasant odour on the fabric, colourfast, organoleptically appealing and as well have commendable quality of sticking to fabrics to avoid crocking (Finar, 1973). Crocking is the rubbing off of dyes from fabric; an indication that the dye is not well absorbed or firmly attached to the fabric. Grayness or crystals of dyes on fabric's surface implies uneven or un-level dyeing which is unacceptable to clothing and fabric consumers. Fabric refers to a flexible material made up of a network of natural or manufactured fibres formed by any of weaving, knitting or other fabrication methods (Vanderhoff et al., 1985). Cotton fabric is processed from cotton plant, a natural fibre, and treated in this study to test the organoleptic quality of dye extracted from roselle calyces.

Roselle calyces have been reported to possess large pigment content and some dye attributes (Technical Centre for Agricultural and Rural Co-operation ACP-EU, 2006), which may be due to the presence of some phytochemicals. Phytochemicals are chemical compounds or bioactive non nutrient compounds that occur in plant and some of the plants are beneficial to human in health and disease prevention. Saponin, Phytate, carotenoid, polyphenols, lutein, flavonoids and tannin among others are phytochemicals in plants which contribute to the colour of plants and other organoleptic attributes of plants from which natural dyes of plant origins are extracted (Win and Swe, 2008).

Organoleptic attributes of a dye are the qualities of a dye that can be seen, touched or felt, perceived or smelt and therefore involve the senses of sight, touch or feel and smell. The colour, texture, odour and evenness of shade or level dyeing are all components of organoleptic attributes of a dye. Colour is an aspect of visual experience (Websters Collegiate Encyclopedia, 2000). The colour of a dye in fabrics or clothing is an important factor in the choice and selection of textiles and clothing items and accessories. Hue, value, chroma or intensity are aspects of colour (Johnson and foster, 1990; Marshal et al,, 2000). Hue is the wavelength reflected from a material. Different wave lengths indicate different hues and approximately 150 hues can be detected in the visible spectrum (Kolender, 2013). Hue is the name of a color family (red, blue, green) and may be warm or cool. Warm hues are red, yellow, orange as found in the sun rays and fire while cool hues are those found in water (blue, green and violet) (Johnson and Foster, 1990). Value is the lightness or darkness of a colour while Chroma or intensity explains the purity of a color expressed as the strength or weakness, dullness or brightness or the degree of saturation of a color. High chroma in colours makes a colour pure, strong, brilliant and saturated and is thus preferred in the choice of dye source than low chroma in colours which makes the colour mute, weak, grayed and dull and less acceptable in the dyeing of fabrics. Johnson and Foster (1990) emphasized that each hue in the color wheel is presented at its fullest and purest chroma or intensity meaning that the colour is at its greatest saturation and brightness; thus can be said to be at the peak of its brilliance. Texture is a sensory impression involving touch and sight (Marshal et al., 2000). Different textures absorb light differently and can change the colour of fabrics. The same dyes applied on different textures produce different colours. The visual aspect of texture is perceived by the eye because of the degree of light absorption and reflection on the surface of the material and can be hard or soft, rough or smooth, hot or cold. Such impressions are the result of sensory impression understood by sight and other sense organs (Bartley, 1996; Hobbs and Rush, 1997). The tactile, feel or 'hand' aspect of texture in fabrics includes the coarseness, softness or crispness and rigidity and are often influenced by the type of dye used in fabric colouration.

Fabric dyeing and printing coloration techniques are vital aspects of clothing and textile components of Home Science programme. Fibre, yarn, fabric and garment dyeing, printing and their variations are indispensable career opportunity or job oriented areas that equip students with relevant entrepreneurial skills that prepare them for the world of work. Dyes are very crucial instructional materials or raw consumables required for teaching and learning and for skill acquisitions in the coloration techniques. No fabric colouration can be successfully carried out without one form of dye or the other

Currently, much emphasis is placed globally on natural dyes because they are biodegradable and eco-friendly. The devastating effect of the environmental pollution and health hazards caused by some non-biodegradable and carcinogenic synthetic dyes could be minimized by exploitation and utilization of natural dyes. Natural dyes are commonly used in the cosmetic industry due to no side effects, their UV protection and anti-aging properties. Extraction of roselle dye will contribute to ensuring

*Corresponding author. E-mail: ozougwusu@yahoo.com. Tel: +2348033318006.

sustainable supply of dye for effective teaching and learning of textiles and clothing and other fields that utilize dyes. Indigenous or home dyers, small and medium scale clothing and textiles industries may increase the volume of clothing and textiles production to generate income thus reducing unemployment, poverty and associated crimes.

It has been observed that the local supply of dyes as important processing chemicals in textile and clothing sector are low and scarce and fail to meet the need of the sector (Onwualu, 2006), many of the synthetic dyes imported into the country are eco unfriendly and pose threat to human life. Presently, in Nigeria, funding for many schools is a great challenge and the inability of the schools to purchase instructional materials and consumables including dyes in sustainable supply poses threats to practical work. Practical exercises are often skipped or stalled and students will not acquire the needed skills that will promote entrepreneurship. Consequently, students may graduate without acquiring the necessary practical skills in clothing and textile programme which could launch them into relevant clothing related entrepreneurial activities. This is a problem. About 143 unexplored dye yielding plants including roselle are locally available (Jansen and Cardon, 2005). These however, have remained largely unexplored. It then becomes necessary to evolve workable ways of sourcing the dyes which students can use for practical work locally.

The general objective of the study was to evaluate the acceptability of cotton fabric treated with dye extracted from roselle (*Hibiscus sabd*tariffa) calyces based on its phytochemical composition and organoleptic attributes.

Specifically, the study sought to:

1. Assess the phytochemical constituents of roselle calyces for carotenoids, flavonoids, lutein, polyphenol and tannins.
2. Evaluate the organoleptic attributes of cotton fabric treated with dye extracted from roselle calyces.
3. Determine the acceptability of cotton fabric treated with roselle dye extract.

Hypothesis

One null hypothesis was tested by the study at 0.05 level of significance:

$H_0$1: There is no significant difference in the mean responses of lecturers and post graduate students on the acceptability of cotton fabric treated with dye extracted from roselle calyces.

MATERIALS AND METHODS

The study was carried out at the University of Nigeria, Nsukka, (UNN) Enugu State, Nigeria using quasi experimental research design. The experimental aspect of the study was carried out in the Analytical laboratory of Home Science, Nutrition and Dietetics Department to determine the phytochemical constituents while assessment of the organoleptic attributes was carried out in the Clothing and Textile laboratory of Home Economics Unit of Vocational Teacher Education Department (VTE), all at the University of Nigeria, Nsukka. The study population comprised of 41 evaluators made up of 17 lecturers and 24 Postgraduate students purposively sampled from the following Departments: Vocational Teacher Education (VTE), Home Science, Nutrition and Dietetics, Pure and Industrial Chemistry, Fine and Applied Arts, all at the University of Nigeria, Nsukka. These departments offer courses relating to dye production or/ and utilization. The lecturers and postgraduate students (who also teach or work with dyes in their various establishments) are in a better position to give accurate evaluation of the organoleptic quality of dye produced from roselle calyces. They are also co-consumers. The detail of the lecturers who were part of the study population was as follows:

1. Seven home economics lecturers from VTE department;
2. Six lecturers from Home Science, Nutrition and Dietetics department;
3. Two lecturers from the Department of Pure and Industrial Chemistry and
4. Three lecturers from Fine and Applied Arts Department UNN. This gave a total of 17 lecturers from the four departments (Source; Departmental records).

The postgraduate (PG) students were also drawn from the respective departments as for the lecturers. The detail of the population of this category was: 12 PG students from (Home Economics Unit) VTE Department:

1. Eight PG students from the department of Home Science, Nutrition and Dietetics, UNN.
2. Two PG students from the department of Pure and Industrial Chemistry and
3. Two PG students from Fine and Applied Arts Department, all from UNN (Source; Departmental Registers 2014 academic session).

Red dry roselle calyces, Aluminum sulphate ($AlSO_4$) (alum), sal soda, ferrous sulphates, methanol. Other materials and equipment used include: Cotton (100%), distilled water, heater, thermometer, rubber hand gloves, Thomas Willey milling machine, stainless and plastic bowels, spoons, laboratory blender, desiccators and freezer. Their uses were explained below alongside the procedure of the work.

Procedure

Red dry roselle calyces (4 kg) were collected from Enugu and further dried under room temperature (40°C for 40 min) in the Green House to enhance quick and smooth milling. The calyces were milled into fine powder using Thomas Willey milling machine. The roselle powder was then kept under room temperature and used within seven days for both chemical analysis of the phytochemical constituents and dye extraction for cotton fabric dyeing and acceptability evaluation.

Quantitative determination of phytochemical compositions of roselle calyces

Carotenoid was determined by gravimetric method as described by Harborne (1973): About 3 g of powdered roselle calyces was homogenized in methanol using a laboratory blender. A 1:10 (10%) solution of methanol was used. The homogenate was filtered to obtain the initial crude extract. Ether (20 ml) was added to the filtrate to take up the carotenoid content. The sample was mixed up very well in a separating funnel and 20 ml of distilled water was added. The ether layer was recovered and then evaporated to dryness at low temperature (35-50^oC) in a vacuum desiccator. The dry extract was then saponified with 20 ml of ethanoic potassium hydroxide (KOH) and left over night in a dark cupboard. The next day, the carotenoid were taken up in 20 ml of ether and then washed with two portions of 20 ml distilled water. The carotenoid extract (ether layer) was dried in a desiccator and then treated with a light petroleum (petroleum spurt) and allowed to stand in a freezer (-10°C). The next day, the precipitated steroid was removed by centrifugation and the carotenoid extract was evaporated to dryness in a weighed evaporation dish, cooled in a desiccator and weighed. The weight of carotenoid was determined and expressed as a percentage of the sample weight.

Flavonoid determination was done using gravimetric method as described by Boham and Kocipai-Abyazan (1994). About 10 g of roselle calyces sample were extracted repeatedly with 100 ml of 80% methanol at room temperature. The whole solution was filtered through Whatman filter paper No 42 (12.5 mm). The filtrate was later transferred into a crucible and evaporated to dryness over a water bath and weighed to a constant weight.

Lutein analysis was carried out using the diethyl ether spectrophotometric method as described by Nurhidayati and Irianty (2012). About 1 g of the powdered roselle calyces was treated with 30 ml of acetone containing 0.1 ml Magnesium Carbonate ($MgCo_3$) in a glass beaker with constant stirring for 16 h. This was filtered and the residue re-extracted with 20 ml acetone until the filtrate became clear. Then 10ml of 50% Potassium Hydroxide (KOH) was added and the filtrate KOH mixture seated on a hot plate for 10 min. The solution was put in a separating funnel and 30 ml diethyl ether and 20 ml water added. The aqueous layer was discarded while the ether layer was recovered and measured in a spectrophotometer at 445 nm against a blank diethyl ether.

Polyphenols determination was by spectrophotometric method according to Association of Official Analytical Chemists (AOAC) (2005). The fat free sample of powdered roselle calyces was boiled with 50 ml of ether for the extraction of the phenolic component for 15 min. 5 ml of the extract was pipetted into a 50 ml flask, and then 10 ml of distilled water was added. 2 ml of ammonium hydroxide (NH_4OH) solution and 5 ml of concentrated amylalcohol were also added. The samples were made up to mark and left to react for 30 min for colour development. This was measured at 505 nm.

Tannin analysis was done using spectrophotometric method according to Van-Burden and Robinson (1981). A 500 mg sample of roselle calyces was weighed into a 50 ml plastic bottle. 50 ml of distilled water was added and shaken for 1 h in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtrate was pipetted out into a test tube and mixed with 2 ml of 0.1 M Iron III Chloride ($FeCl_3$) in 0.I N Hydrogen Chloride (HCl) and 0.008 M Potassium ferrocyanide ($K_4(FeCN)_2$. The absorbance was measured at 720 nm within 10 min. If the absorbance was not measured within 10 min, Iron III Chloride ($FeCl_3$) may become too thick or heavy and refuse to be read in the spectrophotometer.

Mordanting cotton fabric

To pre-mordant the fabric, 25 g of cotton fabric (40"x40") was scoured thoroughly in warm water with detergent three times to remove all finishes. Aluminum sulphate ($AlSO_4$) (alum) of 6.25 and 0.5 g of sodium carbonate ($NaCo_3$) were dissolved in 1 L of heated distilled water. The wet scoured cotton fabric was gently immersed and thoroughly stirred so that it opened out in the solution. It was heated at 80°C for 1 h and allowed to cool overnight in the solution then squeezed off excess water.

Dye extraction and fabric dyeing

Extraction of dye from roselle calyces was done using boiling method as described by Kolender (2003). A portion of 80 g rosselle powder was dissolved in 160 ml distilled water in the ratio 1:2 (W/V) and heated at 80°C for 20 min and allowed to cool. The heated portion was filtered with 0.5 mm mesh (Particle size) to collect the dye liquor. The mordanted cotton fabric was immersed in the roselle dye bath for 1hr at a temperature of 80°C using the contemporary plain dyeing method. The colour was modified with additional 0.25 g ferrous sulphate ($FeSO_4$) added to the dye bath. The dyed fabric was taken out, washed and dried under a shade.

Evaluation of the organoleptic attributes and acceptability of roselle dyed cotton fabric

The rating of the organoleptic attributes and acceptability of the dyed fabric was done by a panel of 41 evaluators using a set of 41 copies of the OAAE instrument. The instrument comprised three sections. Section A sought personal data of evaluators including the department and status (lectuerer/student, male/female, married/single). Section B dealt with organoleptic attributes of cotton fabric treated with roselle dye including colour hue, value, chroma, textures of sight and feel, odour and levelness or evenness of shade of roselle dye on dyed cotton fabric. A 5-pont scale with real limit of numbers was used to take decisions on the organoleptic attributes where mean in the range of 5 in the scale represents the highest identified attribute and mean in the range of 1 indicate the least attribute as shown below using table of real limit of numbers (Table 1).

Section C evaluated the acceptability of the organoleptic attributes of roselle dyed cotton fabric by the evaluators. Mean 3 and above on any attribute of the roselle dyed cotton fabric indicate that such attribute is accepted by evaluators as good attribute of a dye on dyed fabric while mean below 3 shows that a particular attribute is not accepted by the evaluators. The table of real limit of numbers below was used to take decisions on the acceptability of the roselle dyed fabric by the evaluators.

Table 2 show real limit of numbers for evaluating the acceptability of roselle dyed cotton fabric. The rating was done in a single session and the evaluators' mean rating responses were collated for data analysis.

Data analysis

Data were analyzed using descriptive statistics (Percentages, mean and standard deviation). Mean 3.00 and above indicate positive and accepted organoleptic attribute whereas mean below 3.00 indicate negative and unaccepted organoleptic attribute. The t-test statistic was used to test the stated null hypothesis. Null was accepted when P-value is less than or equal to 0.05 ($P \leq 0.05$) and rejected when P-value is greater than 0.05 ($P > 0.05$) significant level.

RESULTS

1. The following phytochemical constituents were present in roselle calyces per 100 g; carotenoid (1.96%), flavonoid (0.02%), lutein (0.03%), polyphenol (0.12%), tannin (0.09%) (Table 3).
2. Identified organoleptic attributes include: Roselle

Table 1. Roselle dye attributes and range of means for taking decisions.

Roselle dye	Range of means for taking decisions				
Range of means	5.00-5.90	4.00-4.90	3.00-3.90	2.00-2.90	1.00-1.90
Colour Hue (Maroon)	Very warm	Warm	Fairly warm	Cool	Very cool
Value	Very light	Light	Fairly light	Dark	Very dark
Chroma/Brightness	Very bright	Bright	Fairly bright	Dull	Very dull
Textures of sight	Very smooth	Smooth	Fairly smooth	Rough	Very rough
Textures of feel	Very soft	Soft	Fairly soft	Crisp/coarse	Very crisp
Odour/Smell	Very pleasant	Pleasant	Odourless	Offensive	Very offensive
Evenness of Shade	Very even	Even	Fairly even	Uneven	Very uneven

Table 2. Real limit of numbers for evaluating the acceptability of roselle dyed cotton fabric.

Range of mean	Decision
5.00-5.90	Very highly accepted (VHA)
4.00-4.90	Highly accepted (HA)
3.00-3.90	Averagely accepted (AA)
2.00-2.90	Unaccepted (UN)
1.00-1.90	Highly unaccepted (HU)

Table 3. Percentage of carotenoid, flavonoids, lutein, polyphenol and tannin phytochemical contents of roselle calyces per 100 g (N=3).

Constituents	X_1	X_2	X_3	X_4	SD	SE
Carotenoid	**2.0285**	**1.8997**	**1.9518**	1.9600	0.0282	*0.0200*
Flavonoid	**0.0168**	**0.0180**	**0.0171**	0.0173	0.0000	0.0000
Lutein	**0.0259**	**0.0249**	**0.0257**	0.0255	0.0035	0.0025
Polyphenol	**0.1211**	**0.1207**	**0.1242**	0.1220	0.0014	0.0010
Tannin	**0.0877**	**0.0875**	**0.0873**	0.0875	0.0049	0.0035

X_1 X_2 X_3 = Mean± SD of three determinations, X_4 = grand mean, SD = standard deviation, SE= standard error of mean.

colour is maroon with fairly warm hue, fairly light value, fairly brilliant chroma, smooth and fairly soft texture, odourless and even shade (Table 4).

3. All the roselle dye attributes on treated cotton fabrics were accepted by both categories of evaluators. There were no significant differences (P>.05) in mean responses of the lecturers and postgraduate students on acceptability of the organoleptic attributes of mcotton fabrics treated with roselle dye at 0.05 significant level (Table 5).

Data in Table 3 shows that of all the phytochemical constituents of roselle calyces determined, carotenoid had the highest quantity present (1.96%), followed by polyphenol (0.12%), tannin (0.09%) and lutein (0.03%). The least phytochemical constituent present was flavonoid (0.02%).

Data in Table 4 reveal that all the organoleptic attributes of roselle dyed alum mordanted cotton fabrics were rated highly by both the lecturers and postgraduate students as shown by the grand mean and standard deviation scores of each attribute. Colour hue when cotton fabric is mordanted with alum mordant was maroon and was fairly warm (3.69±0.73). Value was fairly light (3.59± 0.54). Chroma was fairly bright (3.63±0.57), and textures of visual perception and feel were smooth and fairly soft respectively (4.12±0.57 and 3.85±0.82). Roselle dye was odourless (3.68 ± 0.72), and shade of colour was even (4.02±0.58). The roselle dyed cotton fabric attribute that attracted the highest rating as shown by the grand mean of both categories of evaluators was texture of sight (4.12 ± 0.58) while the lowest rated attribute was colour value (3.59 ±0.54).

All the organoleptic attributes of roselle dyed cotton fabric were rated from averagely to highly acceptable by

Table 4. Mean responses of lecturers and postgraduate students on the organoleptic attributes of cotton fabric treated with roselle dye.

S/N	Attributes	X_1	SD_1	X_2	SD_2	X_3	SD_3
1	Hue (Maroon)	3.59	0.793	3.79	0.658	**3.69**	**0.726**
2	Value	3.35	0.702	3.83	0.381	**3.59**	**0.542**
3	Chroma	3.88	0.485	3.37	0.648	**3.63**	**0.567**
4	Texture (Sight)	4.24	0.562	4.00	0.590	**4.12**	**0.576**
5	Texture (Feel)	3.82	0.951	3.88	0.680	**3.85**	**0.816**
6	Odour/ smell	3.53	0.800	3.83	0.637	**3.68**	**0.719**

X_l, Mean of lecturers; n_l, Number of lecturers; SD_l, standard deviation for lecturers; X_2, mean of postgraduate students, n_2, number of postgraduate students; X_3, grand mean; SD2, standard deviation of postgraduate students; N-Total number of respondents SD_3. standard deviation for grand mean.

Table 5. Mean rating responses and t-test results of lecturers and postgraduate students on the acceptability of the organoleptic attributes of cotton fabric treated with roselle dye.

S/N	Attributes	n_1=17		n_2=24		N_T=41		T-cal	P-val
		X_1	SD_1	X_2	SD_2	X_3	SD_3		
1	Hue (Maroon)	4.12	0.600	4.08	.502	**4.10**	**0.551**	0.198	0.844
2	Value	4.00	0.612	3.88	.612	**3.94**	**0.612**	0.644	0.523
3	Chroma	3.71	0.849	3.96	.462	**3.84**	**0.656**	-1.225	0.228
4	Texture (Sight)	4.08	0.502	4.12	.600	**4.10**	**0.551**	0.198	0.844
5	Texture (Feel)	3.88	0.322	4.21	.415	**4.05**	**0.369**	-2.684	0.011
6	Odour/smell	3.24	0.752	3.21	.415	**3.23**	**0.584**	0.147	0.884
7	Evenness of shade	4.00	0.707	4.00	.417	4.00	0.562	0.000	1.000
	Average	3.86	0.640	3.90	.470	3.89	0.555	-.557	0.581

X_l, Mean of lecturers; N_l, number of lecturers; SD_l, standard deviation for lecturers; X_2, mean of postgraduate students; N_2, number of postgraduate students; Df, degree of freedom (39); SD_2, standard deviation of postgraduate students; T-cal, T calculated.grand mean; SD_3, standard deviation for grand mean.

both categories of evaluators. This could be seen by their different grand mean values ranging from 3.23 to 4.10 in Table 5. For instance the roselle maroon colour hue, texture of visual perception and feel as well as evenness of shade of roselle dye on cotton fabric were all highly accepted with mean scores in the range of 4 as shown in the table. While colour hue was warm (4.10±0.55), value was fairly light (3.94±0.61), chroma was fairly bright (3.82±0.65), textures on visual perception and feel of the treated cotton were smooth and soft (4.10±0.55 and 4.05±0.36 respectively). Roselle dyed cotton fabric was odourless (3.23±0.58) and attained level dyeing or evenness of shade of colour.

Hypothesis 1

There is no significant difference in the mean responses of Lecturers and Postgraduate students on the acceptability of the organoleptic attributes of cotton fabric treated with roselle dyes. The calculated p-value of 0.58 of the total or cluster mean in Table 5 is greater than 0.05 (p>.05) significant level at degree of freedom 39. This result indicates that there is no significant difference in the mean responses of lecturers and postgraduate students on the acceptability of the organoleptic attributes of cotton fabric treated with roselle dye.

Decision rule

If p-calculated is greater than 0.05 null hypothesis will be accepted while alternative hypothesis will be rejected but if p-calculated is equal or less than (p<0.05) 0.05, null hypothesis will be accepted while alternative hypothesis will be rejected. Null is therefore accepted at 0.05 level of significance since the p-value (0.581) is greater (P≥ 0.05) than 0.05 level of significance.

DISCUSSION

The study findings on the phytochemical quantitative estimation of the crude yield of roselle calyces reveal that it is high in carotenoid (1.96%), flavonoid (0.02%), lutein (0.03%), polyphenol (0.12%), tannin (0.09%). Flavonoid (0.03%) was the lowest phytochemical constituent of roselle calyces amongst those studied. This finding supports Rao and Seshadri (1942) in Ali et al. (2005), who isolated flavonol glycocides from the flowers of *H. sabdariffa* and observed that the content of flavonol glycosides in the calyces was very low and that these compounds were to be found primarily in the flower petals.

Regarding the organoleptic attributes of roselle dyed cotton fabric, seven attributes were identified. For instance the colour hue was maroon and fairly warm, value was fairly light, chroma was fairly bright, textures on visual perception and feel were smooth and fairly soft respectively. Roselle dyed cotton fabric was odourless and attained level dyeing or evenness of shade of colour. Win and Swe (2008) emphasized that the presence of tannin, carotenoids, polyphenols, lutein flavonoids among other phytochemicals in plants contribute to the colour of plant. Roselle is a plant. These phytochemical constituents were present in roselle calyces in considerate quantities as shown by the study findings and could have probably contributed to the organoleptic attributes of roselle dye on cotton fabric studied. This could be seen by their different mean ratings ranging from 3.23 to 4.10. Colour hue (Maroon) was the attribute most accepted concurrently by both lecturers and PG students though with different but closely related mean (4.12 ±.60 and 4.08±.50, respectively). This finding supports Apparel Search Company (2009) and Lao Silk and Craft (2009), that natural dyes produce wide range of interesting colours with different mordants. Roselle dye yielded interesting maroon colour on 100% cotton fabric using alum as mordant. When 100% cotton fabric is mordanted with other mordants other than alum, roselle dye could produce a different colour or more. They stressed that by using natural plant dyes, natural dyeing experts find beautiful colour springs from unlikely places and by using traditional recipes with new variations, artisans, individuals and home makers can transform roots, leaves, bark, berries and seeds of plants in their home backyards into natural dyes to produce colours and designs on textiles and garments that appeal to people aesthetically and in fashion. The very high score on texture of feel of the roselle dyed cotton fabric by the lectuuers confirms Chengaiah et al. (2010), observation that natural dyes produce soft texture, feel or “hand” in fabric and give cooling sensations that are calmatives and revitalize the skin as many of the plants natural dyes possess remarkable antimicrobial activity and are currently used in cosmetics industry due to no side effects but possess UV protection and anti-aging properties (Chengaiah et al., 2010). The finding also supports Ashis and Agarwal (2009), who emphasized that natural dyes produce uncommon soothing and soft shades compared to synthetic dyes.

In preliminary investigations, an ‘Analysis of fabrics treated with dyes extracted from roselle calyces was conducted. The colour fastness of 100% cotton, stone silk (60% silk, 40% polyester) and polyester (100%) fabrics mordanted with alum and treated with dyes extracted from roselle calyces was explored. Roselle dyed cotton fabric was found to show reasonable fastness to sunlight, acid perspiration and crocking but relatively poor to washing and generally non fast to stone silk and polyester prototypes. The findings also revealed no significant differences in the effect of boiling, steeping and solvent extraction methods used but significant differences existed in the effects of mordant or dye fixatives (alum, tannic and citric acid) used on the colour fatness of the roselle dyed fabrics. Alum and tannic acid mordants had comparable more positive improvements on the colour fassness of the prototype dyed fabrics studied.

Conclusion

Findings from the present study showed that roselle calyces contain reasonable quantities of carotenoid, polyphenol, tannin, lutein and flavonoid phytochemical constituents in descending order of magnitude per 100 g. The identified organoleptic attributes of cotton fabric treated with raselle dye which were acceptable by the evaluators were attributed to the phytochemical constituents of the dye. With the results of the preliminary study on the colourfastness of roselle dye on 100% cotton fabric using alum mordant, roselle dye is a promising dye that can serve as a useful colourant to 100% cotton fabric when mordanted with alum. This has much implication to textiles and clothing students and teachers, home dyers, medium and large scale textile and clothing industries, agriculturists and textile chemists.

RECOMMENDATIONS

1. Textile and clothing teachers at all levels of education in Nigeria should encourage their students to explore plants in their environment for dye extraction and utilization through classroom experiments and research development efforts. The exploitation of the plant dyes will contribute greatly to availability of dyes in sustainable supply for academic, industrial and entrepreneurial purposes.
2. Home makers and artisans or individuals who practice fabric dyeing of any sort should be educated through workshops, seminars, conferences and community meetings on the need to explore the plants in their communities or even their home backyard gardens including roselle dyes using the processes and procedures in the study. These should be explored for entrepreneurship in fabric colouration and other

purposes.

3. The large scale textiles and clothing industries should appreciate the findings of this study by using the identified roselle dye. The industries should find ways of sourcing and improving the quality of the dyes through their textile chemists.

4. More cotton and roselle plants should be cultivated by the agriculturist to ensure sustainable supply of the plant dyes and cotton fibres.

5. The Federal and State Governments through their various agencies and programmes such as skill training and acquisition Programmes should assist individuals, rural dwellers, medium and small scale dyeing enterprises owners or those who would want to embark on dyeing related enterprise using natural dyes by way of funding and bursary awards.

6. A replication of the study could be carried out using other mordants that are human and environmental friendly other than alum used in this work to study their effects.

Conflict of Interests

The authors have not declared any conflict of interests.

REFERENCES

Ali BH, Wabel NAl, Blunden G (2005). Phytochemical, pharmacological and toxicological aspects of Hibiscus sabdariffa L: A review. Phytotherapy 19:369-375.

Apparel Search Company (2009). Dye definition for the clothing and fabric industry. http://www.apearelsearch.com/definition/Dye/dye

Ashis KS, Agarwal P (2009). Application of natural dyes on textiles. Indian J. Fibre Textile Res. 34:384.

Association of Official Analytical Chemists AOAC (2005). Official methods of analysis, 17th Edition. Washington DC.

Bartley SH (1996). Visual perception: Concise Encyclopedia of Psychology. In: Cosiri RJ, Alerbach AJ New York. John Willy & Sons P 298.

Boham BA, Kocipai-Abyazan C (1994). Flavonoids and condensed tannins from leaves of Hawaiian Vaccinium vaticulatum and V. calycinium. Pac. Sci. 48:458-463.

Chengaiah B, Rao KM, Kumar K, Alagusundaram M, Chetty CM (2010). Medicinal importance of natural dyes - A review. Int. J. Pharm. Technol. Res. CODEN (USA). IJRIF 2(1):144-154.

Finar IL (1973). Organic chemistry, the fundamental principle. UK. Longman Group Limited P 875.

Harborne JB (1973). Phytochemical methods: A guide to modern techniques in plants analysis London. Chapman and Hall pp. 49-188.

Jansen PCM, Cardon D (2005). Plant resources of Tropical Africa 3-dyes and tannins. Wageningen, PROTA Foundations P 11.

Johnson JG, Foster AG (1990). Clothing, image and impact. Cincinnati. Ohio. South Western Publishing Co. P 145.

Kolender C (2003). Chemistry of Dyes http://www.aurasilk.com/info/naturaldying.shtm/.

Marshal SG, Jackson JO, Stanley MS, Kefgen M, Tochie–Specht P (2000). Individuality in clothing selection and personal appearance. New Jersey. Prentice Hall Inc. 18:220.

Onwualu P (2006). Role of research and development in industrial raw material sourcing in Nigeria: 2nd, distinguished lecture of the School of Postgraduate Studies, University of Nigeria, Nsukka. http://www.rmrddc.gov.ng P 22.

Rao SP, Seshadri TR (1942). Isolation of hibiscitrin from the flowers of Hibiscus sabdariffa: constitution of hibiscetin. Proc. Indian Acad. Sci. 15a:48-153.

Schippers RR (2000). African indigenous vegetables. An overview of cultivated species. Uk. National Resources Institute /ACP-EU. P. 119.

The Technical Centre for Agricultural and Rural Cooperation ACP- EU (2006). Spore: EU Tech. Centre Agric. Rural Cooperation Biol. Monthly Bull. 126:7.

Van-Burden TP, Robinson WC (1981). Formation of complexes between protein and tannin acid. J. Agric. Food Chem. 1:77.

Vanderhoff M, Frank L, Campbell L (1985). Textiles for Homes and Peoples. Massachusetts. Gin and Company 14:122, 133.

Websters M (2000). Dye, something used as a coloring matter. Merriam Websters Collegiate Encyclopedia USA. Websters Incorporated P 3.

Win ZM, Swe MM (2008). Purification of natural dyestuff extracted from mango bark for application on protein fibres. World Acad. Sci. Eng. Technol. P 46.

academicJournals

Vol. 11(33), pp. 3082-3089, 18 August, 2016
DOI: 10.5897/AJAR2016.11177
Article Number: 4E2F93C60033
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Wheat cultivars agronomic characteristics and technological quality of flour depending on the row spacing

Tatiane Pauly[1]*, Antonio Carlos Torres da Costa[1], Maurício Antonio Pauly[1], José Barbosa Duarte Júnior[1], Sheila Cordeiro Silva-matte[1] and Tiago Roque Benetoli da Silva[2]

[1]Universidade Estadual do Oeste do Paraná, UNIOESTE. Campus de Marechal Cândido Rondon. Centro de Ciências Agrárias, Programa de Pós-Graduação em Agronomia. Rua Pernambuco, 1777 – Caixa Postal: 91. CEP: 85960-000 – Marechal Cândido Rondon PR (45) 3284-7911, Brazil.
[2]Departamento de Ciências Agronômicas, Universidade Estadual de Maringá – UEM, Campus de Umuarama. Estrada da Paca s/n, CEP: 87500-000, Bairro São Cristóvão, Umuarama, PR, Brazil.

Received 2 May, 2016; Accepted 26 July, 2016

Proper handling of the wheat crop is crucial to obtain the full expression of the genotype potential. Among the various management techniques, the spatial distribution of plants and the disposal of the same plant in agriculture deserve attention. It is known that the distribution may alter the productivity; however, it is uncertain, the technological changes it might entails. With this in view, the aim of this study was to evaluate in two wheat cultivars, characteristics of agronomic interest and the technological quality of flour depending on the spacing. The experimental design was a randomized block with four replications in a factorial 4 × 2. The first factor refers to the spacing: 20, 28, 36 and 40 cm and the second factor refers to the cultivars:CD 150 and BRS Tarumã. The experiment was carried out in Santa Tereza do Oeste, western region of Parana state, in May 2011. The yield and grain moisture were reduced with increased spacing of 3.98 and 26.18%. The hectolitre weight, thousand grain weight, the moisture of the flour, ash content dry basis, the falling number, and flour color were not affected by increasing spacing. Cultivar CD 150 presented lighter color, higher weight hectolitre, lower ash content and fewer drop from the BRS Tarumã.

Key words: BRS Tarumã, CD 150, *Triticum aestivum.*

INTRODUCTION

The price paid to the farmer for wheat produced is directly linked to the technological qualities it has. Thus, to plant a crop expecting to get good productivity and quality, it is necessary to evaluate and determine which soil conditions will be most suitable; what the climatic condition of the region is; which is the most suitable

*Corresponding author. E-mail: tatiane.pauly@yahoo.com.br.

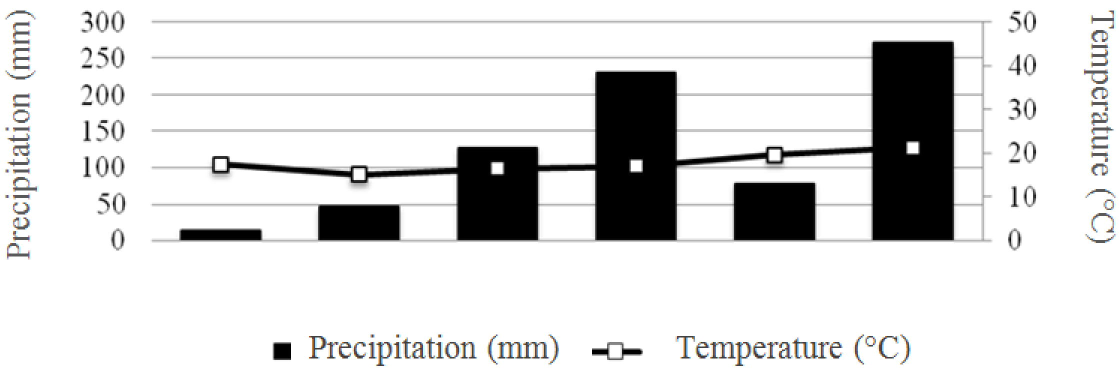

Figure 1. Average monthly temperature and precipitation chart, from 14/05/2011 to 29/10/2011. Santa Tereza do Oeste, Paraná, Brasil. Source: Sistema Meteorológico do Paraná - SIMEPAR (2013).

cultivar; what cultural practices will be adopted; the number of plants per unit area as well as the spacing between lines; and finally, the collection and evaluation of flour quality. These assumptions which lead to productive crops are also crucial in influencing the nutritional and technological characteristics of wheat flour. The technological quality of flour after the grain has been grinded directly influences the quality of the final food product which it is used for. There is therefore an increase in quality demand by customers from the mills (Zardo, 2010).

According to Fontoura (2005), after choosing the best genotype for the desired purpose, proper crop management is crucial for the genotype to reach its full potential. Among the various management techniques, the spatial distribution of plants and their arrangement deserve special attention. When creating a microclimate with certain characteristics, such conditions can affect productivity, either by encouraging direct competition between plants, or by indirectly favoring or disfavoring the development of disease-causing pathogens. However, there are no reports linking the spatial distribution of plants and the technological quality of flour. The objective of this study was to evaluate the characteristics of agronomic interest and the technological quality of flour of two wheat cultivars based on the spacing.

MATERIALS AND METHODS

The experiment was conducted under field conditions during the growing season of 2011, in Santa Tereza do Oeste, western region of Parana state, Brasil (25°03'08"S, 53°37'59"W and 749 m asl). The soil of the experimental area is classified as eutrophic red latosol (EMBRAPA, 2006) and the climate is Cfa according to Köppen classification.

The experiment was conducted in a managed area of tillage on corn stover. The area soil from from 0.0 to 20.0 cm presented the following: pH ($CaCl_2$): 5.8; C: 34.0 g dm^{-3}; P (Mehlich 1): 10.3 mg dm^{-3}; H + Al: 3.97 cmolc dm^{-3}; K +: 0.22 cmolc dm^{-3}; Ca^2 +: 5.4 cmolc dm^{-3}; Mg 2+: 4.0 cmolc dm^{-3}; CTC (pH 7.0): 13.59 cmolc dm^{-3}; base saturation (V%): 70.8%. Climate rainfall data and monthly average temperature during the growing season, were obtained from the meteorological station of Simepar (Figure 1).

The experimental design was a randomized block design with four replications in a factorial 4 x 2 design. The first factor refers to the spacing: 20, 28, 36 and 45 cm while the second factors were cultivars CD 150 and BRS Tarumã. Each plot had 4 x 2.5 meters totaling 10 m^2. The plots were composed of different numbers of rows; 20, 14, 11 and 9 in accordance with the spacing described above. However, the length was the same for all plots (2.5 m) and plant density per meter remained between 75 and 80 plants per meter. For the evaluations, useful areas of each plot were used, neglecting two extreme lines of the portion and 50 cm from each end portion, the useful area of each experimental plot varied according to the line spacing adopted: 8 9, 7.9, 7.6 and 7.2 m^2 respectively to the line spacing described above).

The NPK fertilizer was applied during seeding and this was done based on the result of soil analysis. This followed the techniques established by the Brazilian Commission indications of Wheat Research and Triticale for the state of Paraná, 2011. The fertilizer was applied in the following distribution: 300 kg ha^{-1} of 08-20-20 formula, corresponding to 24 kg ha^{-1} N, 60 kg ha^{-1} of P_2O_5 and 60 kg ha^{-1} K_2O.

The cultivars BRS Tarumã and CD 150 were used. To carry out seeding, the seeds were treated with imidacloprid insecticide and fungicide triadimenol at doses 0.13 and 0.27 L per 100 kg seed respectively. Sowing was carried out on 14/05/2011, with 85 seeds per meter in order to obtain the density of approximately 75 to 80 plants per meter. There was no soil preparation, because the area had been used in direct seeding system for 8 years in succession cultures planting corn in summer and oatmeal in the winter. Desiccation of the experimental area was performed 15 days before sowing with the use of glyphosate at a dose of 480 g a.i. L^{-1}. The seedling in both cultivars emereged six days after sowing. Nitrogen fertilization was performed at the beginning of tillering which was 35 days after emergence (DAE). It was used as top dressing, and urea broadcasting was done with doses of 30 kg N ha^{-1}.

Harvest was done on 17/09/2011 and 29/10/2011. By this time, the plants were at the point of harvest. The CD 150 cultivars were harvested on 122 days after seedling emergence while the BRS Tarumã was harvested on 162 days after seedling emergence. The agronomic characteristics evaluated were: grain yield (PRO), thousand grain weight (MMG), grain number $spike^{-1}$ (NGE), spike number m^{-2} (NEM) and plant height (Al). The grain weight test (PH) was measured for quality. For the quality of the meal, the following factors were considered: (NQ), color (L *, a * and b *), ash on a dry basis (CIN), flour moisture (UF) and grain moisture (UG).

The harvested wheats were measured in order to determine the weight (PRO) kg ha^{-1}. At the same time, the plant's height was evaluated at three random points per plot. The plant was measured

Table 1. Summary analysis of variances: plant height (AL), spike number m^{-2} (NEM), number of spike^{-1} grain (NGE), thousand grain weight (MMG) and productivity (kg ha^{-1}) (PRO). UNIOESTE / PPGA, Santa Tereza do Oeste, Paraná, Brasil, 2011.

Source of variation	GL	Squares average				
		AL	NEM	NGE	MMG	PRO
Spacing	3	0.792^{ns}	25732.08*	2.54^{ns}	0.63^{ns}	632590.53*
Cultivate	1	1123.14*	15400.12*	98.0*	56.34*	992992.78*
Spacing * Cultivate	3	2.767^{ns}	464.04*	3.92^{ns}	1.03^{ns}	10335.53^{ns}
Block	3	2.477	26.08	2.54	1.60	83493.78
Error	21	3.691	59.12	5.14	0.77	62762.80
CV (%)		2.78	12.19	6.91	3.22	12.53

No significant ns. * Significant at 5% level by the F test (p <0.05).

from the ground to the stalk apex including the awn. The number of grains per spike-1 (NGE) was determined with an average of 10 randomly sampled spikes per plot. The number of spike m-2 (NS) was determined by a frame with an area of 1 m2 released at random in the useful area of each plot.

After the threshing quality parameter tests were performed, the hectoliter weight (pH) was determined with scale marks Dalle Molle®, corrected to 13%, according to manufacturer's methodology. The thousand grain weight (MMG) was determined by the Seed Analysis Rules (Brazil, 2011).

Wheat conditioning (pre-grinding humidification) was performed according to the 26-10A - AACC (2000) method and wheat flour was obtained by grinding the grain mill pilot aid mark Chopin. The color was determined using the method 14-22 - AACC (2000). The grain moisture (UG) and flour (UF) were determined by the method. 44-15A - AACC (2000). The ash content of dry basis (CIN) was measured using the method. 08-01 - AACC (1995). The falling number (NQ) was performed using the method. 56-81B - AACC (2000). The data were subjected to variance analysis by applying the F test at 5% probability.

RESULTS AND DISCUSSION

No significant differences were observed between treatments with respect to spacings (productivity), cultivars (plant height, grain number spike $^{-1}$, thousand grain weight and productivity) and interaction between spacing x cultivar (number of spikes per m^{-2}). The summary of the analysis of variance is shown in Table 1.

The plant height, leaf number, the thousand grain weight and productivity can vary in magnitude depending on the development conditions. When the plant grows in low density, generally it becomes greater than a similar plant at high density, and often has a different morphology. Despite that the wheat plant showed high phenotypic capacity, there was no interaction between spacings of x cultvars and plant height. However, there were significant differences among cultivars (Table 1), and BRS Tarumã showed greater height (74.98 cm) when compared to the cultivar CD 150 that had an average height (63.13 cm), (Table 2). Sources et al. (2000) confirms the results of the spacing, however, the height differs with increase in density in the plantation line.

The number of m^{-2} cultivars of wheat was influenced by the interaction spacing of x cultivar. For BRS Tarumã there was a linear decrease of the spike number m^{-2} in relation to the spacing (Figure 2). The highest average m^{-2} for this cultivar of wheat was obtained at 421.75 spacing of 20 cm, while the lowest average (299, 50) was obtained at a spacing of 45 cm. For the cultivar CD 150, the curve that best fit the points was the quadratic polynomial. According to the data presented in Figure 2, it is observed that for the cultivar CD 150, the largest number of spikes m^{-2} (382.25) was obtained at a spacing of 20cm while the smallest number of spikes (242.25) was obtained at a spacing of 45 cm. Thus, it is evident that the number of grains per area decreases as spacing increases. Among the cultivars, the averages found for BRS Tarumã were higher than CD 150 in all analyzed spacings. The difference found in the number of spike m^{-2} can be explained by genetic differences in the cultivars. The dual purpose capability found in BRS Tarumã demonstrated high ability to tillering compared to cultivar CD 150. Since the quadratic found for cultivar CD 150 shows that there was compensation in the number of spikes m^{-2} (spacings 28 and 36 cm) the issue of wheat was visibly limited to genetic factors of cultivar.

Results obtained by Wendt et al. (2006) and Fontes et al. (1997) corroborates the results of this test on the straight reduction in the number of m^{-2} ears. Gross et al. (2012) and Alvarenga et al. (2009) also observed linear reduction. The number of grains per ear (Table 2) of BRS Tarumã and CD 150 cultivars was not influenced by increase in space; however, there were significant differences between cultivars. The highest average was obtained by cultivar CD 150 (34.56) while BRS Tarumã obtained an average of 31.06.

Results obtained by Fioreza (2011), Sources (1997) and Silva and Gomes (1986) corroborate these findings, however, increase in density resulted in a decrease in the number of grains in the ears. Moreover, Valério et al. (2008) obtained similar results with increased spacing; however, there was no effect on plant density. The thousand grain weight of BRS Tarumã and CD 150 was not affected by the change in spacing (Table 2). The best

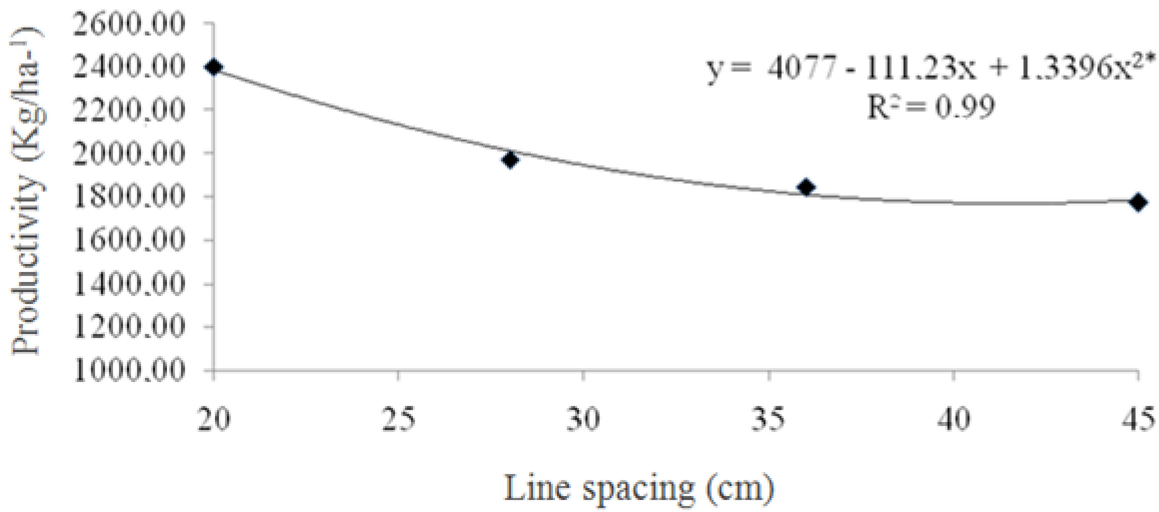

Figure 2. Number of spike m^{-2} wheat cultivars depending on the spacing. UNIOESTE / PPGA, Santa Tereza do Oeste, Paraná, Brasil, 2011.

Table 2. Plant height (cm), number of grains per $spike^{-1}$, thousand grain weight and productivity (kg ha^{-1}) of wheat cultivars depending on the spacing. UNIOESTE / PPGA, Santa Tereza do Oeste, Paraná, Brasil, 2011.

Cultivars	Plant height (cm)	Number of grains per $spike^{-1}$	1000 grain mass	Productivity (kg ha^{-1})
CD 150	63.134^b	34.560^a	28.467^a	1,822.500^b
BRS Tarumã	74.983^a	31.062^b	25.813^b	2,174.812^a
CV %	2.78	6.91	3.22	12.53

Means followed by the same letter in the column do not differ by F test at 5% probability.

average was obtained by cultivar CD 150 (28.467) while BRS Tarumã showed a value of 25.813 (Table 3). The results observed in this study for the thousand grain weight are similar to those obtained by Fontes et al. (1997) and Gross et al. (2012), which showed no correlation between spacing and thousand grain weight. However, the study found reduction of this variable with increase in plant density.

In the 2011/2012 harvest, the wheat yield reached an average of 2399 kg ha^{-1} in the state of Paraná, while the national productivity average was 2672 kg ha^{-1} (Conab, 2012). In this research, the average productivity considering the spacing of 20 cm, was up to 2,400 kg ha^{-1} and, therefore, similar to the Paranamese average for the same crop (Figure 3).

The grain yields were not affected by the spacing between cultivar. However, there were significant differences among cultivars (Table 1), and, BRS Tarumã showed better grain yield (2174.8 kg ha^{-1}) when compared to cultivar CD 150 which showed a productivity of 1822.5 kg ha^{-1} (Table 2).

On the effect of spacing on the productivity of both cultivar grains, there was a quadratic polynomial response, which was a decrease in grain yield as there was increase in the spacing between rows of cultivation. Figure 3 shows maximum productivity of 2401.87 kg ha^{-} in the spacing of 0.20 m and minimum yield of 1773.12 kg ha^{-1} in the spacing of 45 cm. The results show that the wheat plant has a high ability to compensate for voids. The tiller offsets the least amount of plants thus causing changes in other physiological variables which account for grain fillings and such feature is strongly limited by the plants genetic ability to issue tillers.

The results show that although the wheat plant has a high ability to compensate for voids by means of the tiller emission offsetting the least amount of plants and causing changes in other physiological variables, which together, account for the grain filling and such feature is strongly limited by the genetic ability of the plant has to issue tillers. Thus, a significant increase in space relates to the adopted does not imply greater emission of tillers to compensate for production.

Zagonel et al. (2002) and Silva and Gomes (1990) found no effect of density on productivity. Moreover, Provenzi et al. (2012) found no effect of spatial arrangement in grain yield. Since Wang et al. (1997) and Fontes et al. (1997) found no quadratic reduction in productivity, but rather linear reduction. Table 4 shows the values of lightness (L*) and chromaticity coordinates a* and b* of wheat flour samples. It can be seen that the flour of CD 150 showed a greater average value of L* of 90, 44 while BRS Tarumã showed an average value of

Table 3. Summary analysis of color (L *, a * and b *), of the flour obtained from different wheat cultivars depending on the spacing. UNIOESTE / PPGA, Santa Tereza do Oeste, Paraná, Brasil, 2011.

Source of variation	GL	Squares average		
		L*	a*	b*
Spacing	3	0.067370[ns]	0.012886[ns]	0.281504[ns]
Cultivate	1	10.024003*	0.081003*	27.602450*
Spacing * Cultivate	3	0.126686[ns]	0.016436[ns]	0.133308[ns]
Block	3	0.315645	0.005503	0.122246
error	21	0.209800	0.010365	0.374512
CV (%)		0.51	25.59	4.92

No significant ns. * Significant at 5% level by the F test (p <0.05).

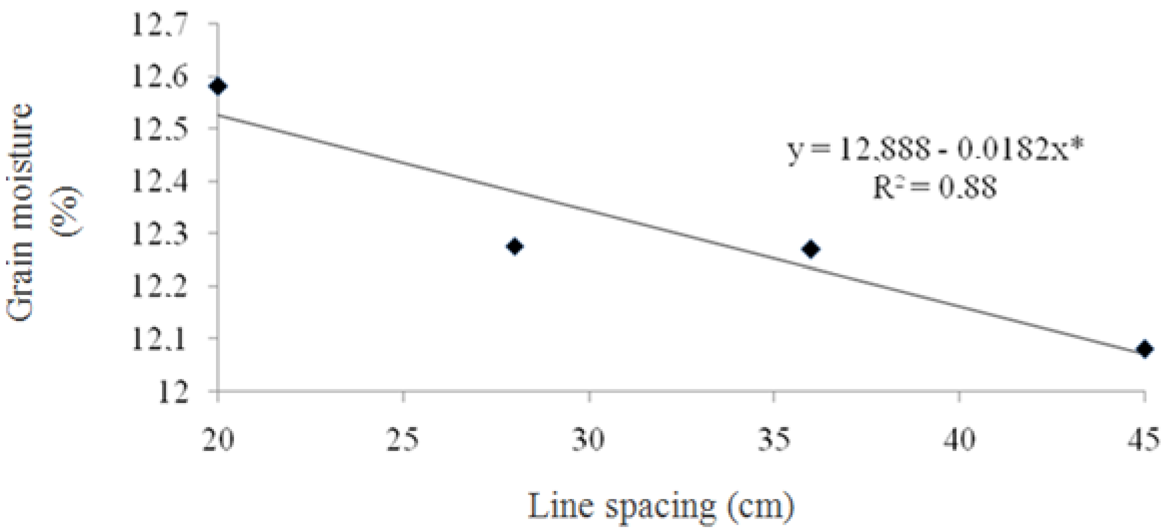

Figure 3. Wheat cultivars' grain yield in different spacing. UNIOESTE / PPGA, Santa Tereza do Oeste, Paraná, Brasil, 2011.

Table 4. Color determined by the CIEL *a *b, through the light parameters (L*) and chromaticity coordinates (a* b*) of wheat cultivars depending on the spacing. UNIOESTE / PPGA, Santa Tereza do Oeste, Paraná, Brasil, 2011.

Genotypes	L*	a*	b*
CD 150	90.439375[a]	0.347500[b]	11.511875[b]
BRS Tarumã	89.320000[b]	0.448125[a]	13.369375[a]
CV (%)	0.51	25.59	4.92

Means followed by the same letter in the column do not differ by F test at 5% probability.

89.32 (Table 4). Cultivar CD 150 showed a higher chromaticity coordinate a*, than BRS Tarumã while the result was reversed for b* chromaticity coordinate (Table 4).

In general, flour is considered white if the L* value is equal to or greater than 93; a* value less than or equal to 0.1 and b* value less than or equal to 8.0 (Gutkoski, 1999) considering these values, cultivar CD 150 is whiter than BRS Tarumã.

In this study, BRS Tarumã and CD 150 cultivars showed different result despite that the pigmentation values handling and environmental conditions which they were exposed to were identical. This result can be explained by the fact that the genetic characteristics of

Table 5. Summary of analysis of variance for hectolitre weight (PH), grain moisture (UG), flour moisture (UF), ash (CIN) and falling number (NQ), wheat cultivars depending on the spacing lines. UNIOESTE / PPGA, Santa Tereza do Oeste, Paraná, Brasil, 2011.

Source of variation	GL	Squares average				
		PH	UG	UF	Ashes	NQ
Espacing	3	0.488^{ns}	0.344*	0.0123^{ns}	0.000004^{ns}	6.7083^{ns}
Cultivate	1	279.13*	0.067^{ns}	0.1116^{ns}	0.002113*	15753.12*
Espacing*Cultivate	3	1.067^{ns}	0.028^{ns}	0.0133^{ns}	0.000021^{ns}	25.2083^{ns}
Block	3	1.902	0.034	0.1210	0.000188	12.3750
Error	21	0.9096	0.076	0.0455	0.000259	47.4702
CV (5%)		1.27	2.25	1.47	2.62	2.05

No significant ns. * Significant at 5% level by the F test (p <0.05).

Table 6. Average hectoliter weight (PH), grain moisture (UG), moisture flour (UF), ash dry basis (%) (AS) and falling number (NQ) of wheat cultivars according to the spacing lines after conditioning of grains. UNIOESTE / PPGA, Santa Tereza do Oeste, Paraná, Brasil, 2011.

Cultivars	PH	UG (%)	UF (%)	NQ	AS (%)
CD 150	77.82^{a}	12.26^{a}	14.456^{a}	314.125^{b}	0.606^{b}
BRS Tarumã	71.92^{b}	12.35^{a}	14.574^{a}	358.500^{a}	0.622^{a}
CV (%)	1.27	2.25	1.47	2.05	2.62

Means followed by the same letter in the column do not differ by F test at 5% probability.

the cultivars were different. Cultivar CD 150 is classified by breeders as improver wheat (flour lighter than other types), while BRS Tarumã is classified as bread type. For this variable, there were no studies found that relate to different row spacing or planting densities. Rodrigues (2012) and Miranda et al. (2011), in managements function adopted for culture, they found different colorations values, which were affected by wheat conditioning method prior to grinding, grinding method and storage of flour and storage time. The test weight of BRS Tarumã and CD 150 was not influenced by increase in spacing, although significant differences between cultivars (Table 5). The ash content of the CD 150 cultivars and BRS Tarumã showed no difference in the interaction spacing x (Table 5). The BRS Tarumã showed a value of 0.62% which is higher than CD 150, which showed a value of 0.61% (Table 6).

According to Pasinato et al. (2009), high ash content can be a result of high extraction, or presence of bran in flour, which is indicated by dark colored flour, less cooking time and an increase in gluten. Dick and Youngs (1988) reported that the ash content as well as moisture, protein and falling number values is strongly influenced by the growing conditions and harvesting of the crop. Such results as the ash content do not corroborate this research because the results were not influenced by the management system of culture, but, by the cultivars. The best average was obtained by cultivar CD 150 (77.8) while BRS Tarumã obtained a value of 71.9 (Table 6). It can be said that the pH difference between the cultivar CD 150 and BRS Tarumã is related to the genetic characteristics of each cultivar.

Sources et al. (1997) corroborate the results found in this study, however, Fontes et al. (2000) found a significant difference in pH which was higher in the spacing of 24.3 cm. Penckowski (2006) corroborates this research and explains that the test weight can be attached to the intrinsic characteristics of each cultivar. According to the F test for grain moisture (Table 6), there was significant difference at 5% probability between treatments with respect to spacings. However, there was no significant difference in the interaction of cultivar x spacing. The observed values as the predicted are shown in Figure 4.

The behavior of grain moisture content compared to the spacing were linear (R2 = 0.88). The presented regression equation shows that on average, there is a decrease of 1.8162% in crop grain moisture for each increase of 1.0 cm row spacing. Under the conditions of this test, the decision was taken when harvesting the beans, in general, met near 12% humidity to avoid production losses. According to the values found, it can be said that the spatial distribution of the crop plants with the added provision in agriculture can lead to microclimate changes between plants. Thus, as the moisture of the grain is influenced by moisture from the ear, it can be said that the microclimate generated between plants due to the increased spacing was

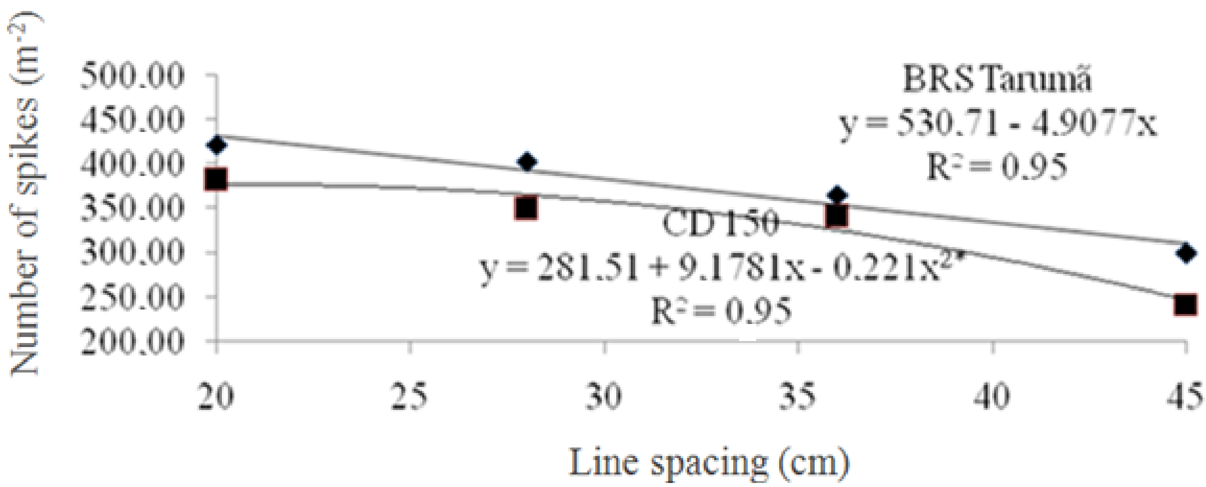

Figure 4. Average wheat cultivars grain moisture due to spacing. UNIOESTE / PPGA, Santa Tereza do Oeste, Paraná, Brasil, 2011.

changed. To date, no studies were found to correlate moisture grain spacing.

The degree of flour moisture after the trial grinding and before performance of color tests, ash content and falling number are shown in Table 6 which shows that the grain conditioning method before grinding was adequate, because humidity levels suffered no significant difference between treatments. Maintaining the humidity was important in the storage of samples and their level did not exceed the level permitted by Brazilian legislation (Brazil, 2011), that is, none of the samples was greater than 15%.

The overall average falling number of BRS Tarumã was 358.50 the 2nd being more than cultivar CD 150, which showed a value of 314.12 seconds (Table 6). According to the classification of cultivars on tolerance to germination in the ear, according to breeders, BRS Tarumã is moderately resistant while the cultivar CD 150 is moderately susceptible to moderately resistant. This may explain the possible differences between cultivars. Increasing row spacing probably modified the microclimate generated between the wheat plants to help reduce moisture in the ear more quickly, thus contributing to the high number of fall in both cultivars.

The interpretation of the falling number, equal to or greater than 300 s are ideal for the production of pasta and bread. Falling number between 250 to 300 s are tolerable, but less than 250 s Number featuring low quality flour and are used for the manufacture of biscuits. These flours have a market price lower than flours marketed for bread and pasta. According to the given parameters, both cultivars decreased number greater than 300 s and can be marketed as flour for bread and pasta. As mentioned earlier, the type of wheat is classified according to pH values (kg / hl) and NQ (seconds). Neglecting the pH values already discussed, the 4 types of wheats conditioned to NQ values are: Type 1: NQ equal greater than 250; Type 2: NQ equal largest 220; Type 3: NQ equal greater 180 and Type 4: NQ equal greater 62. In this test, the genotypes NQ than 250, therefore, the classification of the type is related to the values observed for PH presented earlier. To date, no studies were found relating the number of fall with different row spacings. Overall, this study showed that increasing the spacing does not influence the technological quality of grain and flour produced; however, it significantly reduces the grain yield.

Conclusion

The grain yield and grain moisture were reduced by 26.18 and 3.98%, respectively, with increasing spacing. The hectolitre weight, thousand grain weight, the moisture of the flour, ash content dry basis, the falling number and flour color were not affected by spacing. Cultivar CD 150 presented lighter color (1.24%), higher hectolitre weight (7.58%), lower ash content (2.57%) and higher number of loss (12.38%) in relation to farming BRS Tarumã.

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

The work received financial support from Coordernação de Aperfeiçoamento de Pessoal de Nível Superior - CAPES.

REFERENCES

Alvarenga CB, Sobrinho JS, Santos EM (2009). Comportamento de cultivares de trigo em diferentes densidades de semeadura sob irrigação indicadas para a região do Brasil Central. Biosci. J. 25:98-107.

BRASIL (2011) . Ministério da Agricultura, Pecuária e Abastecimento. Instrução Normativa n° 38, de 30 de novembro de 2010. Regulamento técnico do trigo. Diário Oficial da República Federativa do Brasil, Brasília, DF. 229, 1 dez. 2010. Seção 1.

CONAB (2012). Companhia Nacional de Abastecimento. Custo de produção – Culturas de inverno – Série histórica. Disponível em: http://www.conab.gov.br/conteudos.php?a=1281&t=2.

Dick JW, Youngs VL (1988). Evaluation of durum wheat, semolina, and pasta in the United States. Durum wheat: chemistry and technology. Am. Assoc. Cereal Chem. 13:237-248.

EMBRAPA Trigo: Empresa Brasileira de Pesquisa Agropecuária (2006). Sistemas de Produção: trigo. 269p.

Fioreza SL (2011). Comportamento produtivo do trigo em função da densidade de semeadura e da aplicação de reguladores vegetais. Dissertação (Mestrado em Agronomia-Agricultura). Universidade Estadual Paulista, Botucatu. P 78.

Fontes JR, Cardoso AA, Sousa MA, Cruz CD (2000). Relação do espaçamento e da densidade de semeadura com o rendimento de grãos e outras características agronômicas do trigo. Rev. Ceres 47:1-13.

Fontes JRM (1997). Efeito de espalhamentos e densidades de semeadura sobre o rendimento de grãos e outras características agronômicas do trigo. Rev. Ceres 44:249-252.

Fontoura SMV (2005). Adubação nitrogenada na cultura do milho em Entre Rios, Guarapuava, Paraná. Fundação Agrár. Pesqui. Agropec. 94p.

Gross TF, Dias AR, Kappes C, Schiebelbein LM, Anselmo JL, Holanda HV (2012). Comportamento produtivo do trigo em diferentes métodos e densidades de semeadura. Sci. Agrar. Paranaensis 11:50-60.

Gutkoski LC, Antunes E, Roman IT (1999). Avaliação do grau de extração de farinhas de trigo e de milho em moinho tipo colonial. Boletim Ceppa 17:153-166.

Miranda MZ, Guarienti EM, Bassoi MC, Scheeren PL, Silva MS, Caierão E (2011). Qualidade tecnológica de cultivares de trigo da Embrapa indicadas para o Paraná na safra 2011. V Reunião da Comissão Bras. Pesqui. Trigo e Triticale. 5p.

Pasinato A (2009). Oficina sobre trigo no Brasil. Passo Fundo: Embrapa Trigo112b p.

Provenzi FD, Régis B, William D, Antonio BJ (2012). Arranjo espacial de plantas em duas cultivares de trigo. Unoesc & Ciência – ACET. 3:31-36.

Penckowski LH (2006). Efeitos do trinexapac-ethyl e do nitrogênio na produtividade da cultura do trigo. Ponta Grossa. Dissertação (Mestrado em Agronomia) – Universidade Estadual de Ponta Grossa, Ponta Grossa P. 84.

Rodrigues TRD (2012). Características agronômicas, qualidade da farinha e avaliação de genótipos de trigo duplo propósito submetidos a diferentes manejos de corte. Dissertação (Mestrado em Agronomia) – Universidade Estadual do Oeste do Paraná, Marechal Cândido Rondon P. 98.

Silva DB, Gomes AC (1990). Espaçamento e densidade de semeadura em trigo irrigado na região dos cerrados. Pesqui. Agropec. Bras. 25:305-315.

Silva DB, Gomes AC (1986). Efeito da densidade de semeadura sobre o trigo irrigado na região dos cerrados. In: Reunião sobre trigo irrigado, Dourados. pp. 25-31.

Valério IP, Carvalho FIF, Oliveira AC, Machado AA, Benin G, Pedro Luiz Scheeren PL, Souza VQ, Hartwig I (2008). Desenvolvimento de afilhos e componentes do rendimento em genótipos de trigo sob diferentes densidades de semeadura. Pesqui. Agropec. Bras. 43:319-326.

Wang Z, Jinmin FU , Mingrong HE, Yanping YIN, Hongming CAO (1997). Planting density effects on assimilation and partitioning of photosynthates during grain filling in the late-sown wheat. Photosyntetica 33:199-204.

Wendt W, Del Duca LJL, Caetano V (2006). Avaliação de cultivares de trigo de duplo propósito, recomendados para cultivo no Estado do Rio Grande do Sul. Embrapa Clima Temperado, Pelotas. 2p. (Comunicado Técnico, 137).

Zagonel J, Venancio WS, Kunz RP, Tanamati H (2002). Doses de nitrogênio e densidade de plantas com e sem um regulador de crescimento afetando o trigo, cultivar or-1. Ciênc. Rural 32:25-29.

Zardo FP (2010). Análises laboratoriais para o controle de qualidade da farinha de trigo. Dissertação (Mestrado) – Instituto Federal de Ciência e Tecnologia do Rio Grande do Sul, Bento Gonçalves. P. 92.

academicJournals

Vol. 11(33), pp. 3090-3096, 18 August, 2016
DOI: 10.5897/AJAR2016.11305
Article Number: ADA3D4660036
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Use of alternative waste wood substrates in the production of seedlings zinnia

Emmerson Rodrigues de Moraes[1*], Alice Silva Santana[2], Joicy Vitória Miranda Peixoto[3] and Rodrigo Vieira da Silva[1]

[1]Federal Institute of Education, Science and Technology Goiano - Campus Morrinhos, CEP: 75650-000, Morrinhos, Goiás, Brazil.
[2]Federal University of the Border, Campus Chapecó, CEP: 89802-260 Chapecó, Santa Catarina, Brazil.
[3]Institute of Agricultural Sciences, Federal University of the Uberlândia, CEP: 38400-902, Uberlândia, Minas Gerais, Brazil.

Received 7 June, 2016; Accepted 26 July, 2016

Ornamental plants require a substrate that ensures the germination and healthy growth and vigor of the seedlings. Thus, this study aimed at studying the most suitable substrate for the production of zinnia seedlings from waste wood. The substrates evaluated in greenhouse were: Plantmax®; Sawdust (SM); SM + clay soil + sand (1: 2: 1); SM + clay soil + sand (2: 2: 1); SM + clay soil + sand (2: 1: 1); SM + clay soil (1: 1); SM + clay soil (1: 1) + Simple Superphosphate (SS); SM + clay soil (1: 1) + Sulphate Ammonium (SA); SM + clay soil (1: 1) SA + + SS; SM + SS; SM + SA; and SM + SS + SA. The sawdust in proportions 1: 1 (sawdust / clay soil) behaved like an excellent ingredient composition on the substrate to produce seedlings zinnia. The SM + SS provides higher percentages of plant emergence, reaching a rate 30% higher compared to the Plantmax®. The superphosphate associated with ammonium sulfate promoted increment in height of zinnias seedlings. The sawdust associated with superphosphate and ammonium sulfate provide excellent results in substrate composition for plant emergence and establishment of seedlings zinnias.

Key words: Ornamentals, propagation, sawdust.

INTRODUCTION

The species *Zinnia elegans* is an herbaceous plant, annual, of full sunlight, belonging to the Asteraceae family. It is popularly known as zínnia or Captain. It presents flowers of the type daisy simple, folded or frizzy, often used in parks and gardens of tropical and subtropical regions. It is an ornamental plant also cultivated for the production of cut flowers (Szopińska and Politycka, 2016) because of their long-term durability, being suitable, also, for the lawn hedge row and massive in full sun in regions of mild and tropical temperatures (Souza et al., 2011). Since a few years ago, the system for produce seedlings is getting modern and advanced on their technological level, because of the adaptation of new techniques, like protected cultivation, intermittent

*Corresponding author. E-mail: emmerson.moraes@ifgoiano.edu.br.

nebulization, climate control in the greenhouses and the use of modern inputs, such as re-usable containers, sterile and biodegradable substrates. The result is the production of seedlings of quality and on a commercial scale as already occurs in the cultures of coffee, citrus and in some forest and oil species (Oliveira et al., 2011).

The substrate is one of the most important factors on obtaining good quality seedlings. The term "substrate" applies to all solid material, which can be natural, synthetic or residual, mineral or organic, different of soil *in situ*, that when is replaced in a container - pure or mixed with other materials - enables the development of the radicular system (Abad et al., 2001). Planting on substrates demonstrate great advance to the cultivation systems on the soil. This system offers advantages such as the more appropriate management of water, the supply of good conditions for seed germination, adequate levels of nutrients. Moreover, it also improves reducing the occurrence of plant health problems that means direct benefits on the yield and the quality of products harvested (Duarte et al., 2011). With the need for sustainable actions and environmental preservation, it becomes necessary and important for the development of substrates with low cost, easy application, long durability and recyclable, or even the development of methods to be used in the conventional cultivation and proving better conditions of soil chemical and physical aspects. The use of discarded material in the environment, like the sapwoods in the formulation of substrates, contributes with reducing it costs and with the reutilization of materials which had no function until this time.

The quality of the sawdust which comes from lumberyards for the production of substrates depends on the species that were processed, time and condition of storage (Paes et al., 2013). The pure sawdust, if used as substrate, can present problems of excess of moisture, being recommended the mixture with other materials considered coarse, like sand, before the cultivation of the plants (Gonçalves et al., 2013). The use of sawdust with very small particles for the confection of substrates may increase the quantity of microspores that will reduce the level of oxygen available for the plants (Neto and Ramos, 2010) and develop anaerobic processes which starts fermentation that produce organic acids (Park et al., 2013). Junior et al. (2011), the chemical characteristics of the sawdust may vary according to the specie of wood, but in general, the sum of nutrients found in the amazon region sawdust is low and the pH varies between 4.0 to sawdust recently processed which was up to 6.0 for the older ones. The sawdust can be found in abundance in lumber regions as occurs with the straw of rice. Both can be used widely in horticulture. Its benefits range from the low cost of obtainment to the chemical, physical and biological characteristics found on it. Araújo et al. (2013) working with different types of substrates in melon plants, observed the same development of seedlings when sawdust is used. They notice also, that the same number of sheets when compared to a substrate was from soil + humus. The objective of this work was to evaluate the effect of substrates made of residues of sawmill or sawdust and fertilizers in the production of seedlings of Z. elegans.

MATERIALS AND METHODS

Location and design

The experiment was conducted in a greenhouse with nursery conditions with 70% of passage of light and approximate average temperature of 26°C and approximate relative humidity of 70%. A completely randomized design with 12 substrates and 5 repetitions in a total of 60 plots was used. Each plot was constituted by 10 polyethylene bags with diameter of 8.0 cm and height of 17.0 cm, totaling 850 cm^{-3} volume. These were placed the substrates and sown three seed of zinnia from plants present in ornamental flowerbeds, in a depth of 1.0 cm. Chapters were harvested when ripe (dry). Seeds were removed manually and stored at ambient temperature (approximately 25°C) for 15 days. Thereafter, the seeds were sown. Through the thinning, only one seedling per purse was left. The irrigation type micro sprinkling consisted of 6.0 mm daily divided into three shifts, being 1/3 at the beginning of the morning, 1/3 at the beginning of the afternoon and 1/3 at the beginning of the evening.

The raw material for the production of different substrates was: washed sand (0.25 to 2 mm), clayey subsoil the oxisol (60% kaolinite clay, type 1:1), bovine manure and sawdust originated from sawmills, which are very common in lumber regions. The sawdust was obtained in sawmills active or deactivated, and was found exposed to time and without use or function. The main specious that had originated the sawdust were: *Carapa guianensis*; *Goupia glabra*; *Jacaranda copaia*; *Dinizia excelsa; Ceiba pentranda*; *Parkia pendula.*

Substrates

The fertilizer dose in the subtrates in which had the addition of SS (Simple Superphosphate) and SA (Sulphate Ammonium) was 0.5 kg of both fertilizers by m³ of substrate. These, together with each substrate were manually homogenized with a hoe until it obtains uniform color. For the production of seedlings, substrates were used in the proportions described in the table footer 1, giving the treatments. Also, in the same table appears the description about the pH, levels of macronutrients and the chemical properties of the substrates after the cut of the seedlings. The Plantmax® characteristics were taken from the product label. The other substrates were analyzed in soil laboratory. These attributes were characterized according to Alvarez V. et al. (1999). It was observed that the levels of phosphorus present in substrates were relatively low. While the levels of potassium present in SM (Sawdust) + clay soil + sand (1:2:1); SM + clay soil + sand (2:2:1); SM + clay soil + sand (2:1:1); SM + clay soil (1:1) + SS; SM + clay soil (1:1) + SA; SM + clay soil (1:1) + SS + SA; SM + SS and SM + SA were low, the SM + SS + SA presented average concentration of "K", SM and SM + clay soil (1:1) which showed a good level of potassium and a commercial substrate was very good.

A very good content of calcium in the commercial substrate was found, medium in SM + clay soil + sand (1:2:1), SM + clay soil + sand (2:2:1), SM + clay soil (1:1), SM + clay soil (1:1) + SS, SM + clay soil (1:1) + SA, SM + clay soil (1:1) + SS + SA, SM + SS, SM + SA and SM + SS + SA was low in SM and SM + clay soil + sand (2:1:1). In the case of the magnesium, a very good content in the

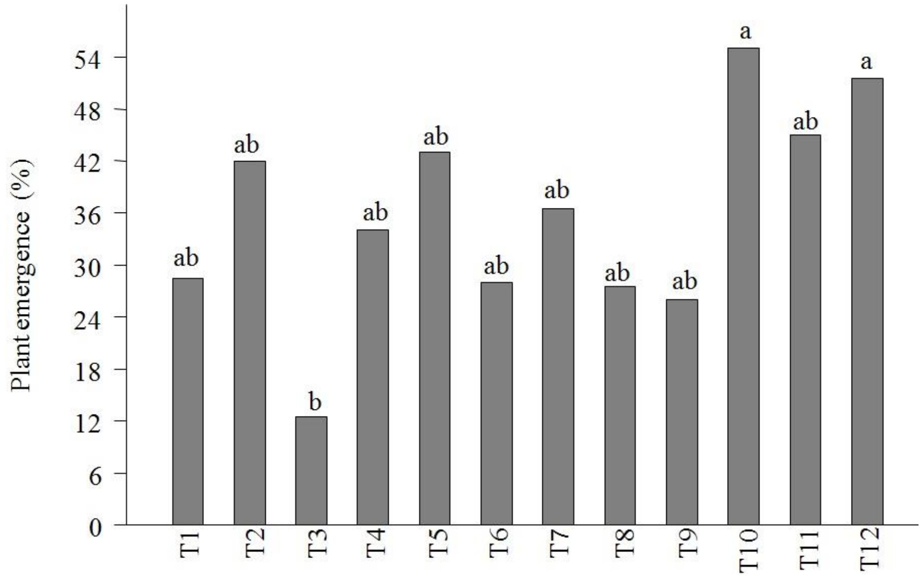

Figure 1. Values of percentage of plant emergence of *Z. elegans* submitted to different types of substrate. T1= commercial substrate; T2= wood sawdust (SM); T3= SM + clay soil + sand (1:2:1); T4= SM + clay soil + sand (2:2:1); T5= SM + clay soil + sand (2:1:1); T6 = SM + clay soil (1:1); T7 = SM + clay soil (1:1) + SS; T8 = SM + clay soil (1:1) + SA; T9 = SM + clay soil (1:1) + SS + SA; T10 = SM + SS; T11 = SM + SA; T12 = SM + SS + SA. Values followed by different letters differ statistically, by F test and Tukey test, at 5% probability. There was normality of the residue by Kolmogorov-Smirnov test and homogeneity of variances by Levene test at 1% probability.

commercial substrate was detected, medium in SM, SM + clay soil (1:1), SM + clay soil (1:1) + SS, SM + clay soil (1:1) + SA, SM + clay soil (1:1) + SS + SA, SM + SA, SM + SS + SA was low in SM + clay soil + sand (1:2:1), SM + clay soil + sand (2:2:1), SM + clay soil + sand (2:1:1) and SM + SS. According to Alvarez et al. (1999) the quantity of aluminum was changeable (Al3+) is low in SM + clay soil (1:1) + SS + SA and very low in the other treatments. The sum of the bases was considered very good in the commercial substrate, medium in SM + clay soil + sand (1:2:1), SM + clay soil + sand (2:2:1), SM + clay soil (1:1), SM + clay soil (1:1) + SS, SM + clay soil (1:1) + ammonium sulfate (SA), SM + clay soil (1:1) + SS + SA, SM + SS, SM + SA, SM + SS + SA was low on SM and SM + clay soil + sand (2:1:1). The potential of acidity (H + Al) was medium in the commercial substrate and low in the other treatments. The cation exchange capacity at pH 7 (T) was very good for commercial substrate Plantmax®, medium in SM + clay soil (1:1), SM + clay soil (1:1) + SS, SM + clay soil (1:1) + ammonium sulfate (SA), SM + clay soil (1:1) + SS + SA, SM + SS, SM + SA and low for SM, SM + clay soil + sand (1:2:1), SM + clay soil + sand (2:2:1), SM + clay soil + sand (2:1:1) and SM + SS + SA. The saturation per basis (V) was very good in the commercial substrate and medium in the rest, while the saturation of aluminum (m) was very low in all treatments. The variables analyzed were the plant emergence (%) for about ten days after the seeding (DAS) and the height of the seedlings at 40 DAS. The seedlings were measured with the aid of a escalímetro graduated (mm). The height of the base of the plant to the apex was measured. After 40 DAS, the seedlings were presented from two to six true leaves, depending on the substrate used, being suitable for transplantation.

Statistical analysis

The results were submitted to analysis of variance (ANOVA) conducted by F test at 5% probability, and the averages compared by Tukey test at 0.05 significance (Ferreira, 2011). ANOVA presuppositions of tests were made concerning the normality of the residuals and homogeneity of variances 1% probability. Principal components analysis (PCA) was performed for emergency and plant height and chemical constituents of the substrates.

RESULTS AND DISCUSSION

In figure 1 are the percentages of plant emergence of *Z. elegans* submitted to many different types of substrates. Observing the percentage of emergence at ten DAS, differences between the substrates was noticed. It can be observed that the substrate SM + SS and SM + SS + SA, were superior to the substrate SM + clay soil + sand (1:2:1). The highest percentage of emergence 51.5 and 55% of seeds in substrates containing SM + SS and SM + SS + SA can be attributed to favorable conditions to germination, such as the availability of water and oxygen in quantities and ideal proportions. According to Souza et al. (2011), there are some factors that interfere in the germination process of seeds in different substrates such as light, oxygen, temperature and water, beside of conditions inherent to the seed as numbness. Another considerable factor on the germination capacity is the genotypic inheritance. Species which are not improved, present high genetic variability in the expression of seed germination and seedling vigor (Martins et al., 2013). The seed treatment against fungi and bacteria also provide

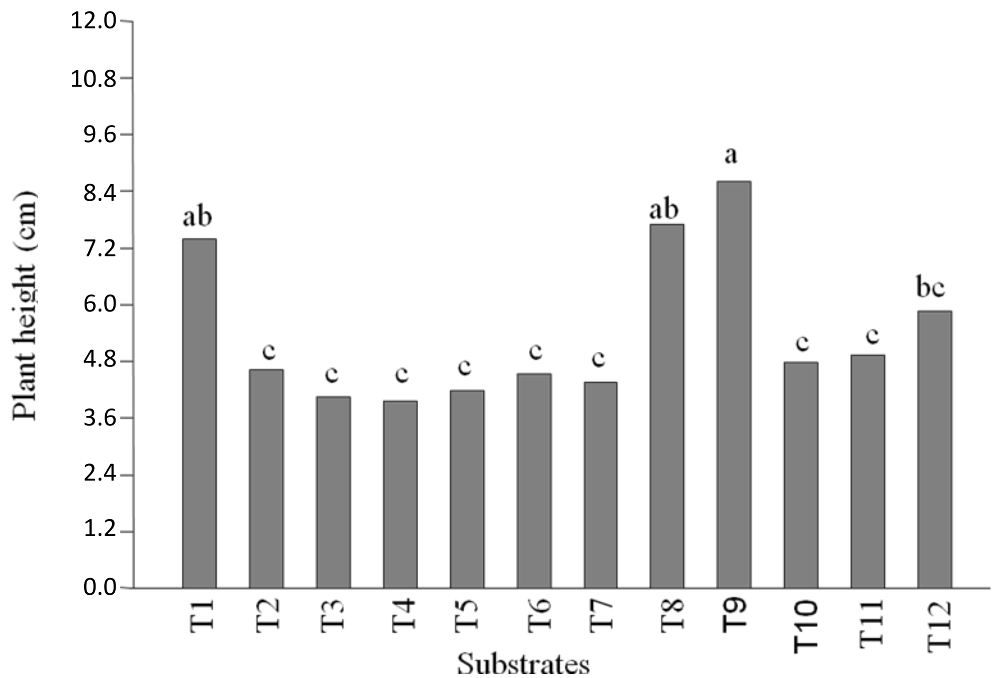

Figure 2. Medium values of plant height of *Z. elegans* submitted to different types of substrates. T1= commercial substrate; T2= wood sawdust (SM); T3= SM + clay soil + sand (1:2:1); T4= SM + clay soil + sand (2:2:1); T5= SM + clay soil + sand (2:1:1); T6 = SM + clay soil (1:1); T7 = SM + clay soil (1:1) + SS; T8 = SM + clay soil (1:1) + SA; T9 = SM + clay soil (1:1) + SS + SA; T10 = SM + SS; T11 = SM + SA; T12 = SM + SS + SA. Values followed by different letters differ statistically, by F test and Tukey test, at 5% probability. There was normality of residue by Kolmogorov-Smirnov test and homogeneity of variances by Levene test at 1% probability.

gains in germination and growth. Szopińska (2014) studied the effects of the treatment with hydrogen peroxide on germination, vigor and seed health of *Zinnia elegans*, observed significant increases in the germination of the seeds treated. Yet, according to the author, the presence of some pathogens influences in the germination of seeds of zinnias between the *Alternaria zinnia,* where, infected samples may present 39.5% of germination. This shows the importance in sanidades seeds to ensure seedlings of good quality.

The availability of water and oxygen is related with the texture of the substrate, which substrates with coarse texture and favor the presence of oxygen, because there are a greater number of macrospores (Gonçalves et al., 2013). In the other hand, substrates with fine texture favor the presence of water, because the number of macrospores is reduced and the number of microspores is increased, this last being responsible for the storage of water (Barreto et al., 2012). According to Ferreira et al. (2008), a good substrate must provide ideal conditions for a higher rate of emergence and favor the growth of the roots. The adequate substrate for germination and plant emergence must present a balance between macro and micro porosity, which promotes a good availability of water and oxygen. For the average height of seedlings (Figure 2) growth of seedlings is observed. In the substrate SM + clay soil (1:1) + SS + SA seedling were performed by plants with height of 8.6 cm higher in 2.8 cm compared to the substrate SM + SS + SA, this being higher than to the others. The height difference between the plants may be related to nutrition available by the substrate allied to its physical characteristics. The simple superphosphate and ammonium sulfate are fertilizers containing phosphorus (18%) and calcium (12%) in the first; and nitrogen (20%) and sulfur (22%) in the second. These fertilizers allied with the macro porosity present in sawdust and microspores present in clay soil, favored the best balance between nutrients, water and air present in the tested treatments. It is worth notice that the nitrogen present in the ammonium sulfate was sufficient to attend the demands of the seedlings, observed because of the good development.

In this experiment, the treatment with commercial substrate Plantmax® was indifferent when compared to treatments SM + clay soil (1:1) + SA, SM + clay soil (1:1) + SS + SA and SM + SS + SA. Frantz (2013) evaluating the efficiency of absorption of phosphorus in different environments by *Z. elegans* showed great growth and rate of flower development of the plants to apply 0.5 mm ($0.5 * 10^{-3}$ mol L^{-1}) which is equivalent to 15.5 mg dm-3 of element. The growth of the plants was reduced as the decrease of the phosphorus applied. The author noted

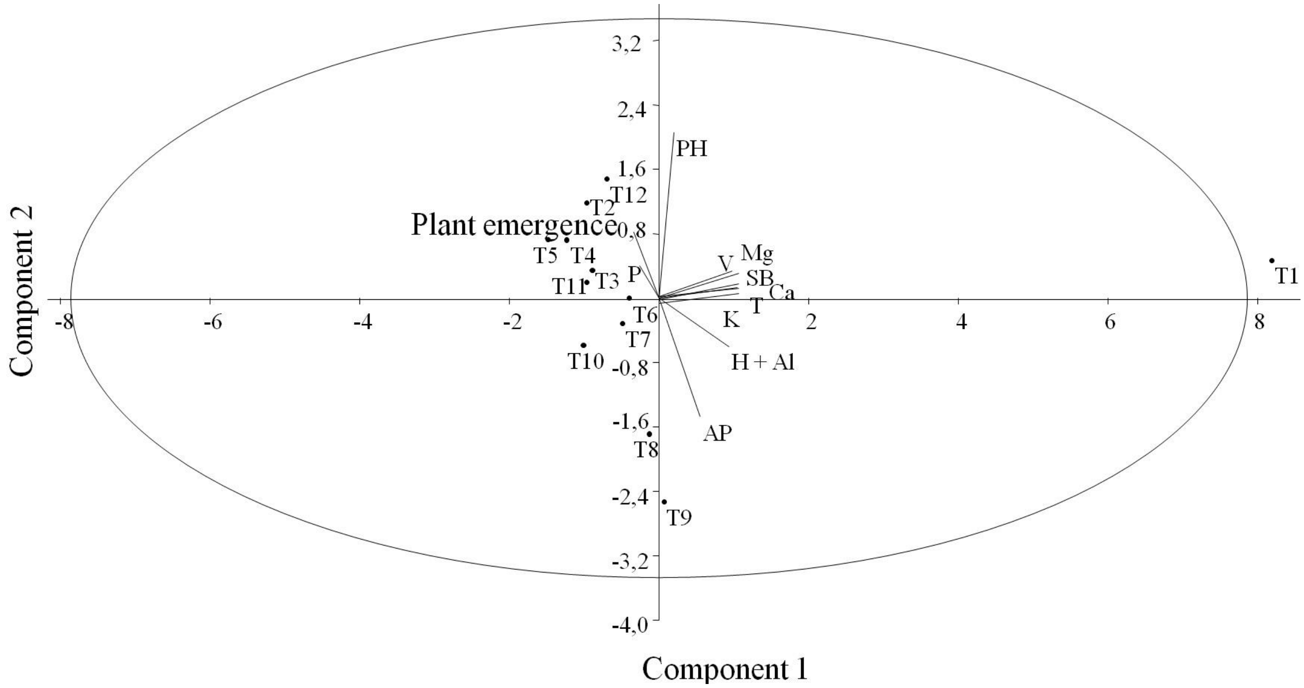

Figure 3. Dispersion of the graphical scores of the principal components one and two in *Z. elegans* evaluated in 11 characters.

that concentrations above 0.5 mm were not beneficial to growth and flowering of the plant. It was found in figure 3, an inverse relation between the variables, plant emergence and phosphorus content with plant height and potential acidity. Probably the different phosphorus contents were present in substrates, their porosity and consequently the balance between the remaining nutrients interfered in the germination and emergence of seedlings reflected at the height at the time of 40 DAPS. The macronutrients calcium, magnesium and potassium correlated among themselves, demonstrating that the development of plants may be degraded because of the detriment of any of these elements.

A correlation was observed between the treatments SM and SM +SS +SA with the plant emergence rate. These treatments had the best emergence rates of 68.7 and 56.0%, respectively, not presenting significant differences between themselves. The sawdust and the sand promoted higher porosity to the substrate by facilitating the germination and the emergence of seedlings. On the other side, substrates containing two parts of clay soil reduced the percentage of plant emergence, reaching only 16%. Hanim et al. (2014), usually, the seeds of annual crops have high germination percentage, reaching up to 95% in the sand when compared to other treatments. These authors obtained 20% of emergency of *Z. elegans* when using soil as substrate, 15% with sand, gravel 5% and 10% to the use of rock dust.

The treatment SM + SS + SA was correlated with the pH, being the substrate with higher pH (6.2) when compared with the other substrates. The treatments SM + clay soil (1:1) + SA and SM + clay soil (1:1) + SS + SA correlate with plant height; these are between the substrates that showed the highest height. Probably the greatest height has a relation with better nutritional balance present in the substrate, being applied sources of nitrogen and sulfur on the first treatment and phosphorus, calcium, nitrogen and sulfur on the second. The height of the plant provides information of their process of growth, being influenced by the nutritional level provided by the nutrient solution and the physical conditions of the substrate like porosity and aeration. The phosphorus is important in all metabolic processes that exist in plants (Schemidt et al. 2010). These authors had observed linear growth in the height of the plants which were submitted to different doses of phosphorus and high correlation of height with other physiological indices like the leaf area (R^2= 0.79), fresh mass of the aerial part (R^2= 0.80) and fresh mass of the root (R^2= 0.73).

No relationship was observed between the commercial substrate Plantmax® (T1) and the analyzed characters. The cultivation with this substrate showed 28.50% of plant emergence and plants with average height of 7.38 cm. This can be explained by better nutritional balance and physical properties presents in this substrate. This showed the highest levels of macronutrients, when compared to the others (Table 1). Castro et al. (2010) emphasize that for the same dose, the source of organic fertilizers used on composting plants can interfere on their growth and development. These authors had notified best results of average height on plants of chrysanthemum for cut when they used the bed of aviary

Table 1. Characterization of substrates about the pH, levels of macronutrients and the chemical properties after the cultivation of the seedlings.

Substrates	pH (H_2O)	Macronutrients						Chemical properties			
		P	K	Ca	Mg	Al	H + Al	SB	T	V	m
		--mg dm^3--		---------cmol$_c$ dm^3-------				------------ % -----------			
Plantmax®	5.9	19.1	290	10.3	6.8	0.0	3.7	17.80	21.50	83	0
T2	6.0	24.3	92	1.0	0.6	0.0	2.2	1.80	4.04	46	0
T3	5.8	17.5	65	1.5	0.4	0.0	1.6	2.07	3.67	56	0
T4	5.6	26.2	55	1.6	0.4	0.1	1.7	2.14	3.84	56	4
T5	5.7	18.9	60	1.1	0.4	0.1	1.6	1.65	3.25	51	6
T6	5.7	15.0	92	1.6	0.6	0.1	2.1	2.44	4.54	54	4
T7	5.5	15.2	63	2.0	0.5	0.1	2.3	2.66	4.96	54	4
T8	5.4	20.2	81	1.7	0.6	0.2	2.3	2.51	4.81	52	7
T9	5.2	19.5	73	2.0	0.5	0.4	2.5	2.69	5.19	52	12
T10	5.2	21.5	55	1.8	0.4	0.2	2.2	2.34	4.54	52	8
T11	5.7	22.9	65	1.3	0.5	0.2	2.5	1.97	4.47	44	7
T12	6.2	18.0	39	1.7	0.6	0.0	1.8	2.40	4.20	57	0

T2= wood sawdust (SM); T3= SM + clay soil + sand (1:2:1); T4= SM + clay soil + sand (2:2:1); T5= SM + clay soil + sand (2:1:1); T6 = SM + clay soil (1:1); T7 = SM + clay soil (1:1) + SS; T8 = SM + clay soil (1:1) + SA; T9 = SM + clay soil (1:1) + SS + SA; T10 = SM + SS; T11 = SM + SA; T12 = SM + SS + SA. SB = sum of bases; T = cation exchange capacity at pH 7.0; V = base saturation; m = saturation of aluminum.

when compared to other sources of organic fertilizer as the bovine and sheep manure. Kenyangi and Blok (2012) found higher efficiency on the length of the aerial part of lettuce using fertilizer, while the peat favored the root growth. The sawdust has promoted excellent physical environment as substrate showing itself similar to all the substrates studied for germination and plant emergence. This has supplied the requirements of porosity and water storage as reported by Silva and Ferreira (2015). As characterized in Table 1, the sawdust has low capacity in the supply of nutrients. So, this did not supply the nutritional demand of seedlings of *Z. elegans*. To assess the development of seedlings of *Eugenia involucrata* Souza et al. (2015) verified smaller development of plants when they use sawdust as substrate, in contrast, the plants showed greater development with the use of commercial substrate Plantmax®. This result is similar with what was found on this study in which the seedlings of *Z. elegans* showed lower plant height when it was used, only the sawdust.

Conclusion

The sawdust associated with simple superphosphate provides the greatest percentage of plant emergence of *Z. elegans*. The simple superphosphate associated with ammonium sulphate promotes an increase in the height of the seedlings of zinnias. The sawdust associated with simple superphosphate and ammonium sulfates are efficient in the composition of substrates, promoting better plant emergence and development of zinnias.

Conflict of interest

The authors have not declared any conflict of interest.

REFERENCES

Abad M, Noguera P, Bures S (2001). National inventory of organic waste for use as growing media for ornamental potted plant production: case study in Spain. Bioresour. Technol. Essex, 77(2):197-200.

Alvarez VVH, Novais RF, Barros NF, Cantarutti RB, Lopes AS (1999). Interpretação dos resultados das análises de solos. In: Ribeiro AC, Guimaraes PTG, Alvarez VVH. Comissão de fertilidade do solo do Estado de Minas Gerais. Recomendações para o uso de corretivos e fertilizantes em Minas Gerais – 5º Aproximação. Viçosa, MG, s.n, 322 p.

Araujo DL , Junior SOM, Silva SF, Andrade JR, Araujo DL (2013). Production of seedlings of melon cantaloupe in different types of substrates. Rev. Verde 8(3):15-20.

Barreto CVG, Testezlaf R, Salvador CA (2012). Dinamic of matricial potencial in substrates of pinus and coconut under action of capilarity. Horticul. Bras. Brasília 30(1):26-31.

Castro AMC, Sato O, Santos KH, Zaparolli RA, Sartori SB, Demetrio GB (2010). Fertilizing with mineral and organic on the development of crisântemo. Semina: Ciências Agrárias, Londrina, 31(1):93-100.

Duarte RF, Sampaio RA, Junior DSB, Silva HP, Parreiras NS, Neves JMG (2011). Inicial growth of seedling of *Acacia mangium* cultivated on fiber mats of coconut containing as substrate sewage sludge. Rev. Árvore, Viçosa 35(1):69-76.

Ferreira DF (2011). Sisvar: a computer statistical analysis system. Ciência e Agrotecnologia, Lavras 35(6):1039-1042.

Ferreira EGBS, Matos VP, Sena LHM, Sales AGFA (2008). Germination of seeds and development of plântulas cockscomb on different substrates. Sci. Agrária, Curitiba 9(2):241-244.

Frantz JM (2013). Uptake efficiency of phosphorus in different light environments by zinnia (*Zinnia elegans*) and vinca (*Catharanthus roseus*). HortSci. Alex. 48(5):594-600.

Goncalves FG, Alexandre RS, Silva AG, Lemes EQ, Rocha AP, Ribeiro

MPA (2013). Emergence and quality of seedlings of *Enterolobium contortisiliquum* (Vell.) Morong (Fabaceae) on different substrates. Rev. Árvore, Viçosa 37(6):1125-1133.

Hanim A, Nazera AAB, Kahar S, Hamdan MN (2014). Effects of different inorganic mulches on seed germination, weed biomass and plant growth. J. Trop. Agric. Fd. Sci. 42(1)29-36.

Junior JOS, Carmello QAC, Sodre GA (2011). Substrate and fertilizing with phosphorus for the production of clone seedling of Cacau. Rev. Bras. de Ciênc. do Solo, Viçosa 35(1)151-159.

Kenyangi A, Blok W (2012). Vermicompost as a component in potting mixes for growth promotion in ornamental plants. Rwanda J. Agric. Sci. 28:53-63.

Martins CC, Bovi MLA, Oliveira SSCD, Vieira RD (2013). Emergência e crescimento inicial de plântulas de Archontophoenix cunninghamiana H. Wendl. & Drude provenientes de sementes de diferentes plantas matrizes. Ciênc. Rural 43(6).

Neto RMR, Ramos CB (2010). Avaliação das características físicas de substratos formulados com resíduos orgânicos para a produção de mudas florestais em tubetes. Pesqui. Aplicada Agrotecnol. Guarapuava 3(2)117-122.

Oliveira AB, Filho SM, Bezerra AME (2011). Tempo de cultivo e tamanho do recipiente na formação de mudas de *Copernicia hospita.* Acta Sci. Agron. Maringá 33(3):533-538.

Paes JB, Diniz CEF, Lima CR, Melo BP, Medeiros NPN (2013). Taninos condensados da casca de angico-vermelho (*Anadenanthera colubrina* var. cebil) extraídos com soluções de hidróxido e sulfito de sódio. Rev. Caatinga, Mossoró 26(3):22-27.

Park JH, Kim SH, Park HD, Lim DJ, Yoon JJ (2013). Feasibility of anaerobic digestion from bioethanol fermentation residue. Bioresour. Technol. 141:177-183.

Schemidt B, Domonkos M, Sumalan R, Biro B (2010). Suppression of arbuscular mycorrhiza's development by high concentration of phosphorus at *Tagetes patula* L. Res. J. Agric. Sci. Washington 42(4):156-162.

Silva LR, Ferreira LG (2015). Desenvolvimento de mudas de melancia sob efeitos de diferentes tipos de bandejas e substratos. Connection Line (12):97-105.

Souza HHF, Bezerra FC, Assis JRN, Ferreira FVM, Silva TC, Crisostomo LA (2011). Produção de mudas de *Zínnia elegans* em substratos a base de resíduos agroindustriais e agropecuários em diferentes tamanhos de recipientes. Revista Brasileira de Horticultura Ornamental, Campinas 17(2):115-120.

Souza PLT, Vieira LR, Boligon AA, Vestena S (2015). Produção e qualidade de mudas de *Eugenia involucrata* DC. em diferentes substratos. Rev. Biociênc., Taubaté 21(1):100-108.

Szopinska D (2014). Effects of hydrogen peroxide treatment on the germination, vigour and health of *Zinnia elegans* seeds. Folia Horticul. 26(1):19-29.

Szopinska D, Politycka B (2016). The effects of hydro- and osmopriming on the germination, vigour and hydrolytic enzymes activity of common zinnia (*Zinnia elegans* Jacq.) seeds. Folia Horticul. 28(1):3-11.

academicJournals

Vol. 11(33), pp. 3097-3103, 18 August, 2016
DOI: 10.5897/AJAR2016.11115
Article Number: E32766960038
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Enzyme activities and gene expression in dry maize seeds and seeds submitted to low germination temperature

Izabel Costa Silva Neta*, Édila Vilela de Resende Von Pinho, Viviane Maria de Abreu, Heloisa Oliveira dos Santos, Danielle Rezende Vilela, Renzo Garcia Von Pinho and Maria Laene Moreira de Carvalho

Department of Agriculture, Seeds Laboratory, Federal University of Lavras (Universidade Federal de Lavras – UFLA), University Campus – P.O. Box 3037, ZIP code 37200-000, Lavras MG, Brazil.

Received 10 April, 2016; Accepted 8 July, 2016

The study was conducted on dry maize seed and seed subjected to sub optimal germination temperature. Four lines of maize seeds were used, two were classified as susceptible to low temperature (44 and 54) and two tolerant to low temperature (64 and 91°C). Water content and germination tests were performed at different temperatures (10 and 25°C) to evaluate the physical and physiological quality of maize seeds. For molecular study, activity of heat-resistant proteins, esterase, superoxide dismutase, catalase and α-amylase enzymes were assessed. Study of AOX1, ZmMPK5, SOD, APX, SAD, ENR and LEA genes with real time polymerase chain reaction (PCR) were carried out. AOX, SAD and LEA genes that are related as tolerant to abiotic stress showed higher expression in line 64, which was classified as tolerant to low germination temperature. The ZmMPK5 gene is associated with increased production of abscisic acid and expressed more in line 54, which was considered as susceptible to low germination temperature.

Key words: *Zea mays,* seed vigor, abiotic stress.

INTRODUCTION

Exposure of plants to low temperatures causes consequences for most biological processes (Allinne et al., 2009), being considered as one of the abiotic stresses that mostly affect growth, productivity and geographical distribution of agricultural crops (Laudencia-Chingcuanco et al., 2011).

Maize is one of the most cultivated cereals in the world, but its productivity is affected, among other factors, by low temperatures. In adequate moisture and temperature of 20 to 30°C, maize seedlings emerge within 4-5 days; however, in low temperature conditions, germination can take up to two weeks or more, compromising the plant stand.

Tolerance to low temperature is a multigenic and a

*Corresponding author E-mail: izabel_agronomia@hotmail.com.

quantitative feature, however, the number of loci that determine this characteristic is not known (Korn et al., 2008), becoming a difficult mechanism to be elucidated by the study of a single gene (Wang et al., 2011). Thus, a better understanding of the genetic basis related to cold tolerance in different maize germplasm can provide suitable information for maize breeding by marker-assisted selection (Fracheboud et al., 2004).

Regarding the seed vigor, studies with molecular techniques have been conducted to elucidate the mechanisms involved in the germination process in abiotic stress conditions such as drought stress (Zhang et al., 2014); saline stress (Wang et al., 2014; Mardani et al., 2014.); high temperature and humidity (Han et al., 2014).

Identification of genes related to characteristics of tolerance to low temperature during germination process may contribute to the development of selection techniques that can reduce the time and effort to assess genetic sources for tolerance to such adverse temperature condition.

Given the above, this work aimed to study the enzyme activities and expression of genes that are known for their importance in plant tolerance to low temperatures, in dry maize seeds and subjected to sub optimal germination temperature.

MATERIALS AND METHODS

The survey was conducted in an experimental area and in the Central Laboratory of Seeds in the Department of Agriculture of the Federal University of Lavras (UFLA), Lavras, MG, at 910 m altitude, 21°14`S latitude and 45°00W longitude. Four lines of maize seeds were used, two being classified as susceptible to low temperature (44 and 54°C) and two tolerant to low temperature (64 and 91°C). In order to evaluate the physical and physiological quality of maize seeds, water content and germination tests were conducted at different temperatures (10 and 25°C). The water content was determined by the oven-dry method at 105°C for 24 h, using two replicates of each line, according to the Rules for Testing Seeds-RAS (Brasil, 2009). Results were expressed as mean percentage (wet basis).

Temperatures of 10 and 25°C in the germination test were used (the germination test at 25°C was carried out only to identify the seed profile). For each temperature, the test was conducted with four replications of 50 seeds, where seeds were germinated in a "Germitest" paper moistened with distilled water in the ratio of 2.5 times the weight of the dry paper. The rolls were placed in plastic bags and kept in a BOD type chamber regulated at temperatures of 10 and 25°C (±3°C). Assessments of normal seedlings were performed at 4, 7, 14 and 21 days after beginning the tests. The results were expressed as mean percentage of normal seedlings from the four replications. It was considered as 'normal seedlings', those who had at least 1 cm of taproot, with two adventitious roots of at least 1 and 1 cm shoot. Percentage of protrusion was also assessed, adopting as standard, the seedlings with at least 0.5 cm of root protrusion. The tests were performed in a completely randomized design and mean comparison test among treatments was taken by Scott-Knott test, at 5% probability, to analyze the results. Analyses were performed in the SISVAR statistical program. For proteomic (except for alpha amylase) and transcripts analyses, dry and soaked seeds at 10°C for 14 days were used.

In order to extract heat-resistance proteins, seeds were ground in a crucible with ice and liquid nitrogen, then a buffer solution was added (50 mM tris-HCL-7.5; 500 mM NaCL; 5 mM $MgCl_2$; 1 mM PMSF) 1:10 (material weight: volume of extraction buffer) and transferred to 1500 µL capacity microtubes. The homogenates were centrifuged at 14,000 rpm for 30 min at 4°C and the supernatant was incubated in a water bath at 85°C for 15 min and then centrifuged again. The supernatant was poured into microtubes, and the pellet discarded. Before application in the gel, the sample tube containing 70 µL extract + 40 µL sample buffer (2.5 ml glycerol, 0.46 g SDS, 20 mg Bromophenol blue and the volume completed to 20 ml of Tris pH 7.5 extraction buffer) were placed in a water bath with boiling water for 5 min (BLACKMAN et al., 1991). 50 mL of protein extract + sample buffer were applied per well in the polyacrylamide gel electrophoresis SDS-PAGE (12.5% separating gel) and 6% (concentrating gel). Electrophoresis was performed at 120 V and gels were stained with 0.05% Coomassie Blue for 12 h and distained in 10% acetic acid solution (Alfenas, 2006).

For extraction of α-amylase enzyme, seeds were germinated in paper roll for 14 days, at a temperature of 10°C. After this period, seeds were cold ground in crucibles with liquid nitrogen. For extraction, 200 mg ground seed powder was suspended in 600 µL extraction buffer (0.2M Tris-HCl, pH 8.0 + 0.4% PVP). Then, there was application of 40 µL protein extract in each well in polyacrylamide gels at 7.5% (separating gel + 1% soluble starch) and 4.5% (concentrating gel). Electrophoresis was performed at 120 V for 6 h.

For catalase, esterase, peroxidase and superoxide dismutase extraction, the following were used: Tris HCL buffer 0.2 M pH 8.0 + (0.1% mercaptoethanol), 250 µL per 100 mg of seeds. The material was homogenized by vortex and kept for 12 h in a refrigerator, followed by centrifugation at 14,000 rpm for 30 min at 4°C. Electrophoresis was performed in polyacrylamide gel 7.5 (separating gel) and 4.5% (concentrating gel), 60 µL sample's supernatant was applied into the gel and electrophoresis was performed at 120 V for 5 h. Gel revelation was according to Alfenas (2006). For extraction of RNA, it was used four seed lines (44, 54, 64 and 91), dry and soaked for 14 days at a temperature of 10°C.

Maize seeds were ground with liquid nitrogen with *Pure Link* RNA *Plant*® (*Invitrogen*) reagent, according to manual instruction. RNA integrity was checked with 1% agarosis gel. RNA quantification was measured in a spectophotometer at 260 and 280 nm wave-length. After nucleic acid extraction, samples were treated with DNA Free DNase (Ambion) to avoid DNA contamination. Protocol was performed according to manufacturer's recommendations. After RNA extraction and purification, cDNA synthesis was performed using High Capacity cDNA Reverse Transcription cDNA® kit (Applied Biosystems), following the manufacturer's recommended protocol.

By gene expression analysis by qRT-PCR, genes were selected based on literature review due to the importance of studies on plant tolerance to low temperatures. Primers were designed using Primer Express 3.0 software (Applied Biosystems) following the search for gene sequences in http://www.ncbi.nlm.nih.gov site (Table 1). Ubiquitin gene was used as endogenous control.

For qRT-PCR, quantitative analysis of gene expression, ABI PRISM 7500 Real-Time PCR (Applied Biosystems) equipment, SYBR Green detection system and cDNA obtained from RNA extracted from the seeds were used. The thermal reaction conditions were 2 min at 50°C, 10 min at 95°C, followed by 40 cycles of 15 s at 95°C and 1 min at 60°C, ending with 15 s at 95°C. Data were collected and stored in the 7500 software program (version 2.0.1). For each reaction, 1.0 µl cDNA diluted 1: 5, 0.2 µL of each primer and 5.0 µl SYBR green Master Mix UDG with ROX (Invitrogen) were used to obtain a final volume of 10.0 µL/sample. Negative controls and melting curves were included in all the analyses. Three biological repetitions were used in three replicates

Table 1. Primer sequences used in qRT-PCR analysis in seed from different maize lines.

Gene	Identification		Sequence 5'----------3'
LEA	Late embryogenesis abundant	F	TGCAGCCTCCTAGTGCTTGTAC
		R	TGGAAGAGGACCTGGGATTG
Ubiquitina	Endogenous control	F	AAGGCCAAGATCCAGGACAA
		R	TTGCTTTCCAGCGAAGATGA
SAD	Putative stearoyl- ACP desaturase	F	GGA TTT CCT CCC TGA CCC A
		R	GTC CAT GCC CTC GTC CAA A
ENR	Putative enoyl-ACP redutase	F	ACCACTACCCGCACAGCAA
		R	CACGGATTTCCTTTCTGATTTTTC
ZmMPK5	Mitogen-activated protein kinase	F	ACTGATGGACCGCAAACC
		R	GGGTGACG AGGAAGTTGG
SOD4	Superoxide dismutase / Antioxidant	F	TGGAGCACCAGAAGATGA
		R	CTCGTGTCC ACCCTTTCC
cAPX	Ascorbate peroxidase / Antioxidant	F	TGAGCGACCAGGACATTG
		R	GAGGGCTTTGTCA CTTGGT
AOX-1	Alternative oxidase	F	GCGAGGAGCAAACAGCAAA
		R	GGCCACTAGTGCGGTCAACT

Table 2. Physiological quality of four maize lines under different germination temperatures.

Line	10°C		25°C
	Prot (%)	Ger (%)	Ger (%)
44	96^{A}	0^{B}	99^{A}
54	78^{B}	0^{B}	96^{A}
64	96^{A}	14^{A}	98^{A}
91	100^{A}	15^{A}	99^{A}
CV	4.98	10.43	1.86

Results followed by the same capital letter in the column do not differ at 5% probability by Scott-Knott test.

techniques for each studied gene and the results were normalized using the threshold cycle (CT) obtained by Ubiquitin reference gene expression.

The CT was determined by the number of cycles in which the fluorescence generated within a reaction crossed the baseline (threshold). The comparative CT method was used. To do so, first, a validation experiment was performed to prove that the efficiency of the target gene amplification and references were similar and very close to 100% (TYAGI; BRATU; KRAMER, 1998). Standard curves for the studied genes were generated from the following dilutions: 1: 5, 1:25, 1: 125, 1: 625 and 1: 3125. This procedure also defined the best cDNA dilutions in each reaction, which was 1: 5.

For each gene, samples of low expression were used as calibrator samples and the method used to measure the relative expression was Relative Standard Curve Method described in the instrument’s procedure handbook (Applied Biosystems). For the quantification of gene expression by real time PCR, values obtained corresponding to the samples' mRNA levels were compared to the values of the control's mRNA levels. After obtaining the raw data, they were analyzed using the 7500 SDS Software (Version 2.0.1). To calculate the expression level of the interest genes, the following was considered: Ct (PCR product exponential growth) of the target gene and the endogenous control, ΔCt = Ct (sample) – Ct (endogenous control) and the ΔΔCt = ΔCt (sample) - ΔCt (calibrator). Then the level of expression was calculated with the formula: RQ = $2^{-\Delta\Delta Ct}$. For graph plot, the SigmaPlot program was used.

RESULTS AND DISCUSSION

The average water content of seeds at the time of testwas 12.6%, with a maximum variation of 1%. Regarding the physiological quality of maize lines seeds used in this study, it could be observed that when the germination test was carried out at 25°C (which is a temperature suitable for maize germination), there was no statistical difference in germination among materials (Table 2). When seeds were germinated at 10°C temperature, stratification of lots in different vigor levels was observed (Table 2). Regarding the germination

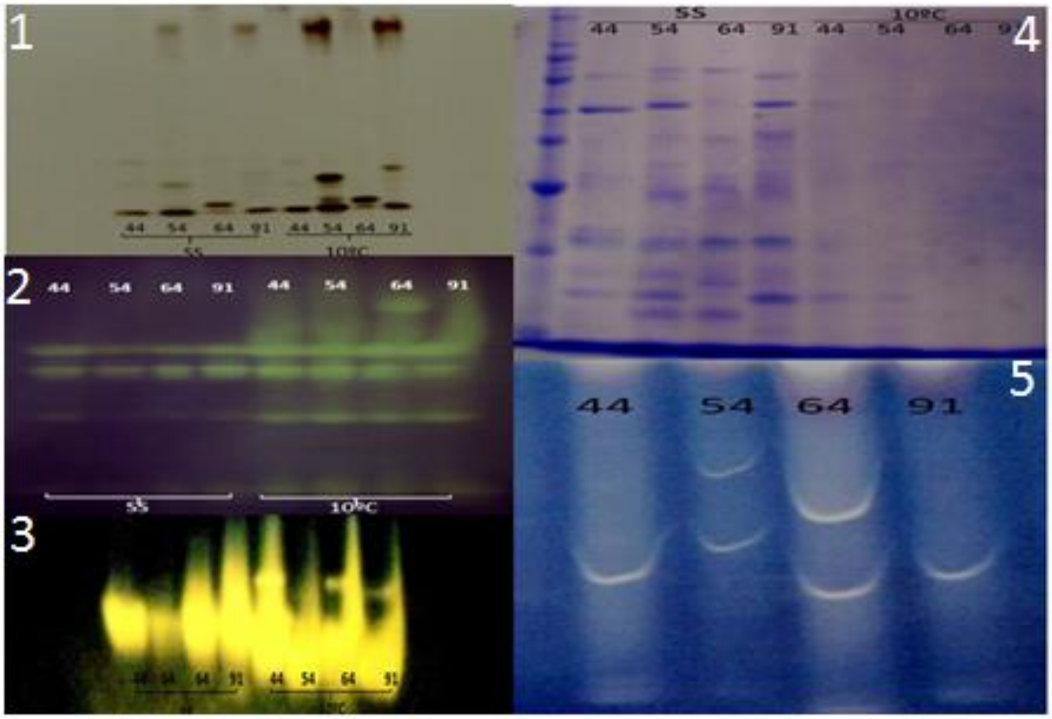

Figure 1. (1) Esterase enzyme activity in dry maize seeds (SS) and subjected to soaking at 10°C for 14 days (10°C); (2) Superoxide dismutase enzyme activity of dry maize seeds (SS) and subjected to soaking at 10°C for 14 days (10°C); (3) Catalase enzyme activity in dry maize seeds (SS) and subjected to soaking at 10°C for 14 days (10°C); (4) Zymogram of heat tolerance proteins in dry maize seeds (SS) and subjected to soaking at 10°C for 14 days (10°C); (5) α-amylase enzyme activity in maize seeds submitted to soaking at 10°C for 14 days.

percentage, lines 44 and 54 showed no seedlings have reached the minimum standard adopted to consider them as normal. With regards to the percentage of protrusion, line 54 was lower than the other lines. This result reflects the effect of genotype on seed tolerance to low germination temperature.

Li et al. (2013), working with induction of cold tolerance in wheat seeds during germination found that when seeds were germinated at 12°C, the germination rate was well below the germination rate of the same materials at 22°C. It is expected that materials more tolerant to low-temperature germination conditions present a well-protected membrane system and an efficient antioxidant defense systems, that is, with increased expression of scavenging enzymes.

Figure 1 shows esterase enzyme activity. It can be seen in this figure that when the seeds were soaked for 14 days at 10°C, seeds from line 54 were the ones that had higher activity of esterase enzyme. The increased activity of this enzyme in these seeds may have triggered a lipid peroxidation, thus contributing to a lower tolerance of this line to cold and thus resulting in low percentages of protrusion and non-occurrence of germination (Table 2). Regarding the superoxide dismutase enzyme (Figure 1), in line 64, there was an expression of an isoform that is not expressed in other materials when seeds were submitted to immersion in stress conditions. Under stress conditions, at low temperatures, reactive oxygen species (ROS) are produced at high levels and may result in damage to DNA, proteins and lipids (Miller et al., 2008). So, the more efficient the antioxidant system, the smaller the damage caused by ROS. The greatest expression of SOD in line 64 may have contributed to this material protrude and originate normal seedlings, even in the temperature of 10°C. Low temperatures affect the germination of hybrid maize seeds because they increase the time for seeds to germinate, reduce the germination percentage and contribute to enhanced superoxide dismutase expression (Farooq et al., 2008).

Regarding the expression of catalase enzyme in the maize lines studied, it appears that for both dry and soaked seeds at 10°C, line 54 showed the lowest expression of this enzyme (Figure 1). Line 54 had a lower percentage of protrusion at 10°C, and the inefficiency of the antioxidant system may have been one of the reasons for this result. Catalase enzyme reduce expression in line 54, even in dry seeds, is an important feature, because it allows the selection of a material during the breeding programs without necessarily making the seeds to germinate.

The increased tolerance to cold may be accompanied by increased expression of specific genes encoding

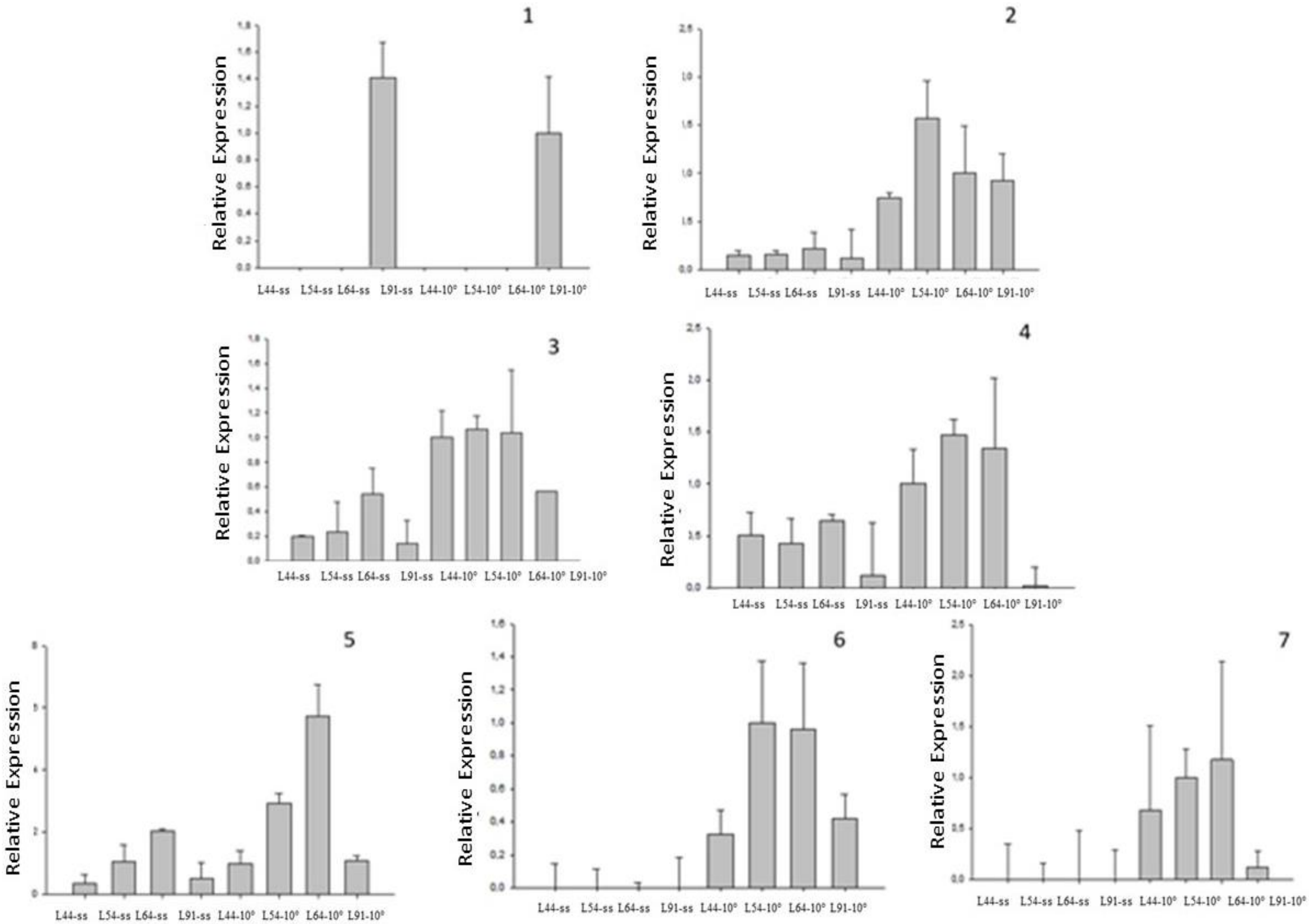

Figure 2. AOX1 gene expression regarding the L64-10º treatment; (2) ZmMPK5 gene expression regarding the L54-10º treatment; (3) SOD gene expression regarding the L54-10º treatment; (4) APX gene expression regarding the L54-10º treatment; (5) SAD gene expression regarding the L64-10° treatment; (6) ENR gene expression regarding the L54-10º treatment; (7) LEA gene expression regarding the L64-10º treatment.

antioxidant enzymes (Baek and Skinner, 2003), plants with increased expression of SOD and other scavenging enzymes designed to increase stress tolerance (Xi et al., 2010). In seeds of line 44, lower expression of heat-resistant proteins (Figure 2) and high protrusion percentage was observed, however after 21 days of soaking, normal seedlings were not observed. Low expression of heat-tolerant proteins may have influenced this result, due to a decreased stability of the membrane system.

In Figure 2, it can be seen that line 64 had the highest expression of α-amylase, so this material was rated as the one with greater tolerance to cold during the germination process, however, line 91, which was also considered to be more tolerant, did not show high activity of this enzyme. These data reflect the complexity of the germination process. In seeds of two recombinant lines which were damaged in controlled conditions, it was possible to identify 65 QTLs for germination energy characteristics, germination percentage, dry root weight and dry shoot weight. However, only five of these QTLs were responsible for representing more than 10% of the phenotypic variation, with highest ratio of 11.92% (Han et al., 2014), the results of this study reinforce the complexity of the germination process. The mechanisms involved in cold tolerance have were also studied through expression of transcripts in a range of species. Figure 1 represents the alternative oxidase gene expression (AOX). It can be seen that only line 64 showed expression of this gene both for dry seed and for soaked seeds at 10°C for 14 days. The expression on dry seeds makes this gene a potential marker for tolerance to low temperature in maize seeds, because it speeds up the selection process. Surge et al. (2006), studying the Arabidopsis response to stress caused by low temperature, found that the AOX activity resulted in attenuation of ROS production. According to Karpova et al. (2002), expression of high levels of AOX in maize occurs due to respiratory failure or membrane depolarization which is responses to cold stress.

Figure 2 shows ZmMPK5 gene expression. Accumulation of ABA and hydrogen peroxide contributes to increased ZmMPK5 expression (Lin et al., 2009). The ZmMPK5 expression was higher in soaked seeds at 10°C than in dry seeds for all the studied lines. Increased expression of this gene was found in seeds soaked for 14 days at 10°C from line 54. This line presented lowest percentage of protrusion when compared with other lines and did not provide seedlings with the minimum characteristics to be considered normal. This susceptibility to low temperature may be due to accumulation of ROS and also higher abscisic acid content, as these two factors may have contributed to greater ZmMPK5 expression. Abscisic acid (ABA) is a phytohormone known to modulate growth of plants in response to stresses (Christmann et al., 2006). ABA regulates important aspects of the plant development, including the initiation and maintenance of seed dormancy. This hormone stabilizes seed dormancy to ensure that germination takes place in a suitable environmental condition (Finch-Savage and Leubner-Metzger, 2006).

Figure 2 presents the expression of superoxide dismutase. This enzyme had higher expression in the dry seeds from line 64, regarding dry seeds of other lines. But for all lines, expression was higher in seeds submitted to cold stress. Seeds from line 91 were those who had lowest expression of SOD. Despite being one of the main antioxidant enzymes, the study of a single gene is not enough to explain the cold tolerance during germination process. Even though line 91 have low SOD expression, it was considered tolerant to low temperatures. This can be explained by the fact that there are several other antioxidant enzymes that could have prevented the production or eliminated the ROS, contributing to the good performance of line 91. In tomato acclimation to cold there is an increase in the NADPH oxidase activity, increase in hydrogen peroxide levels in the cell apoplast and increase in the activity of antioxidant enzymes in an attempt to alleviate the oxidative process and maintain the stability of the membrane (Zhou et al., 2012). Another important antioxidant enzyme is ascorbate peroxidase. The expression of this enzyme is represented in the Figure 2. The expression of this enzyme was lower in line 91, when seeds were soaked at 10°C. This enzyme neutralizes peroxide through ascorbate. For seeds of other lines soaked at 10°C, the expression in the materials was similar. Figure 2 show the expression of genes related to lipid composition of membranes. In *Arabidopsis thaliana*, expression of desaturases (FAD8) was strongly induced by low temperature (Gibson, 1994). Liu et al. (2006) studying the effect of temperature on tomato crops verified that the expression of desaturases (LeFAD7) was induced by stress caused by low temperature (4°C), but inhibited by high temperature (45°C) in leaves. In rice, FAD2 gene seems to be related to stress resistance in plants grown under unfavorable temperature conditions (Shi et al., 2012). Kodama et al. (1995) observed that unsaturated fatty acid is one of the factors involved in tolerance to low temperatures in young tobacco leaves. Figure 2 shows the expression of putative stearoyl-ACP desaturase gene (SAD). Desaturase converts saturated fatty acid into unsaturated. In general, the SAD expression was higher in soaked seeds than in dry seeds in the four studied lines. In dry seeds, greatest expression of this gene was verified in line 64. When seeds were soaked at 10°C, the behavior was the same, and seeds of line 64 had higher SAD expression than other lines. Among the studied materials, line 64 was considered tolerant to low germination temperature. Putative enoyl-ACP reductase (ENR) gene expression is presented on Graph 6. The expression of this gene was higher in seeds soaked at 10°C than in dry seeds and when seeds were soaked, lines 54 and 64 had higher expression of this gene. Gene expression related to LEA protein (late embryogenesis abundant) is represented in the Figure 2. The lower expression of this gene was found in line 91 when seeds were soaked at 10°C. Heat tolerance proteins have been studied as important in plant tolerance to cold stress. Line 91 had high percentage of protrusion at 10°C, however, it did not have high expression of the antioxidant genes studied in this work, nor LEA proteins and desaturase. This result indicates the complexity of cold tolerance characteristics and that studies of few genes are not enough to select materials with tolerance to low temperatures during the germination process. However, this work shows that there are differences in expression between the materials and that some genes related to tolerance to low temperature are expressed more in line 64 which has also been identified as tolerant. AOX, LEA and SAD gene can be mentioned.

Another gene that also had an interesting correlation was ZmMPK5. This gene showed higher expression in line 54, which had the lowest percentage of protrusion at 10°C when compared with the other three lines. Expression of this gene is associated with ABA accumulation, and it is known that abscisic acid is a hormone that negatively affects the germination, being related to seed dormancy process.

So it is possible to suggest that research in the field of molecular analysis focused on seeds, can bring great benefits to understanding the mechanisms involved in tolerance to abiotic stresses and contribute to selection or development of tolerant materials to these stresses. However, studies must be conducted to identify a larger number of genes that can be used to select maize materials with tolerance to low germination temperature.

Conclusions

1. For some studied genes, there was correlation of increased expression with higher tolerance to low temperature. AOX, SAD and LEA genes that are related to tolerance to abiotic stress had higher expression in line 64, after materials were subjected to soaking at 10°C for 14 days.
2. ZmMPK5 gene is associated with increased production of abscisic acid and was expressed more in line 54, which was classified as susceptible to low germination temperature.

Conflict of Interests

The authors have not declared any conflict of interests.

REFERENCES

Alfenas AC (2006). Eletroforese de isoenzimas e proteínas afins: fundamentos e aplicações em plantas e microorganismos. Viçosa, MG: UFV 574 p.

Allinne C, Maury P, Sarrafi A, Grieu P (2009). Genetic control of physiological traits associated to low temperature growth in sunflower under early sowing conditions. Plant Sci. 177:349-359.

Baek Kh, Skinner Dz (2003). Alteration of antioxidant enzyme gene expression during cold acclimation of near-isogenic wheat lines. Plant Sci. 165:1221-1227.

Brasil (2009). Ministério da Agricultura, Pecuária e Abastecimento. *Regras para análise de sementes*. Brasília 399 p.

Christmann A, Moes D, Himmelbach A, Yang Y, Tang Y, Grill E (2006). Integration of abscisic acid signalling into plant responses, Plant Biol. 8:314-325.

Farooq M, Aziz T, Cheema ZA, Hussain M, Khaliq A (2008). Activation of Antioxidant System by KCl Improves the Chilling Tolerance in Hybrid Maize. J. Agron. Crop Sci. 94:438-448.

Finch-Savage WE, Leubner-Metzger G (2006). Seed dormancy and the control of germination. New Phytol. 171:501-523.

Fracheboud Y, Jompuk C, Ribaut JM, Stamp P, Leipner J (2004). Genetic analysis of cold-tolerance of photosynthesis in maize. Plant Mol. Biol. 56:241-253.

Gibson S, Arondel V, Iba K, Somerville C (1994). Cloning of a Temperature-Regulated Gene Encoding A Chloroplast Omega-3 Desaturase From Arabidopsis-Thaliana. Plant Physiol. 106(4):1615-1621.

Han Z, Ku L, Zhang Z, Zhang J, Guo S, Liu H, Zhao R, Ren Z, Zhang L, Su H, Dong L, Chen Y (2014). QTLs for Seed Vigor-Related Traits Identified in Maize Seeds Germinated under Artificial Aging Conditions. PLoS ONE 9(3):e92535.

Karpova OV, Kuzmin EV, Elthon TE, Newton KJ (2002). Differential expression of Alternative Oxidase Genes in maize mitochondrial mutants. The Plant Cell 14:3271-3284.

Kodama H, Horiguchi G, Nishiuchi T, Nishimura M, Iba K (1995). Fatty-Acid Desaturation During Chilling Acclimation Is One Of The Factors Involved In Conferring Low-Temperature Tolerance To Young Tobacco-Leaves. Plant Physiol. 107(4):1177-1185.

Korn M, Peterek S, Mock H, Heyer AG, Hincha DK (2008). Heterosis in the freezing tolerance, and sugar and flavonoid contents of crosses between Arabidopsis thaliana accessions of widely varying freezing tolerance. Plant Cell Environ. 31:813-827.

Laudencia-Chingcuanco D, Ganeshan S, You F, Fowler B, Chibbar R, Anderson O (2011). Genome-wide gene expression analysis supports a developmental model of low temperature tolerance gene regulation in wheat (*Triticum aestivum* L.). BMC Genom. 12:299.

Li X, Jiang H, Liu F, Cai J, Dai T, Cao W, Jiang D (2013). Induction of chilling tolerance in wheat during germination by pre- soaking seed with nitric oxide and gibberellin. Plant Growth Regul. 71:31-40.

Lin F, Ding H, Wang J, Zhang H, Zhang A, Zhang Y, Tan M, Dong W, Jiang M (2009). Positive feedback regulation of maize NADPH oxidase by mitogen-activated protein kinase cascade in abscisic acid signaling. J. Exp. Bot. 60:3221-238.

Liu XY, Yang JH, Li B, Yang XM, Meng QW (2006). Antisense-mediated depletion of tomato chloroplast omega-3 fatty acid desaturase enhances thermal tolerance. J. Integr. Plant Biol. 48(9)1096-1107.

Mardani Z, Rabiei B, Sabouri H, Sabouri A (2014). Identification of molecular markers linked to salt-tolerant genes at germination stage of rice. Plant Breed. 133:196-202.

Miller G, Shulaev V, Mittler R (2008). Reactive oxygen signaling and abiotic stress. Physiol. Plantarum. 133:481-489.

Shi J, Cao Y, Fan X, Li M, Wang Y, Ming F (2012). A rice microsomal delta-12 fatty acid desaturase can enhance resistance to cold stress in yeast and Oryza sativa. Mol. Breed. 29(3):743-757.

Wang YY, Hecker AG, Hauser BA (2014). The APX4 locus regulates seed vigor and seedling growth in Arabidopsis thaliana. Planta 239:909-919.

Wang Z, Wang F, Zhou R, Wang J, Zhang H (2011). Identification of quantitative trait loci for cold tolerance during the germination and seedling stages in rice (*Oryza sativa* L.). Euphytica 181:405-413.

Xi DM, Liu WS, Yang GD, Wu CA, Zheng CC (2010). Seed-specific overexpression of antioxidant genes in Arabidopsis enhances oxidative stress tolerance during germination and early seedling growth. Plant Biotechnol. J. 8:796-806.

Zhang H, Cui F, Wang H (2014). Detection of quantitative trait loci (QTLs) for seedling traits and drought tolerance in wheat using three related recombinant inbred line (RIL) populations. *Euphytica* 196:313-330.

Zhou J, Wang J, Shi K, Xia XJ, Zhou YH, Yu JQ (2012). Hydrogen peroxide is involved in the cold acclimation-induced chilling tolerance of tomato plants. Plant Physiol. Biochem. 60:141-e149.

academicJournals

Vol. 11(33), pp. 3104-3110, 18 August, 2016
DOI: 10.5897/AJAR2016. 11205
Article Number: BF1376360040
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Agronomic characteristics of sorghum in an agroforestry system with Eucalyptus in the semiarid region of minas gerais

Carlos Juliano Brant Albuquerque[1]*, Walyson Silva Soares[2], Regina Maria Quintão Lana[2] and Gilberto Cardoso dos Santos[3]

[1]Instituto de Ciências Agrárias, Universidade Federal de Minas Gerais, ICA-UFMG Av. Universitária, no. 1000 Bairro Universitário, Montes Claros-MG, CEP: 39404 -547, Brasil.
[2]Instituto de Ciências Agrárias, Universidade Federal de Uberlândia, ICIAG-UFU Av. Amazonas, s/n. Bloco 2E, Umuarama, Uberlândia-MG, CEP: 38400-902. CP. 593, Brasil.
[3]Universidade Estadual de Montes Claros, Endereço: Av. Reinaldo Viana, 2630, Bairro Bico da Pedra, Caixa Postal 91. Janaúba-MG Brasil.

Received 10 May, 2016; Accepted 20 July, 2016

The evaluation of agronomic characteristics of crops is important because they may be influenced by the height, density and spatial arrangements of eucalyptus in an agroforestry system. These silvicultural characteristics may interfere with height and productivity of crops between the rows of the tree component due to shading, competition for water and nutrients. The objective of this study was to evaluate the effect of the assessed site on some agronomic characteristics such as height, grain yield and effective yield of BRS 655 forage sorghum (*Sorghum bicolor* (L). Moench) with different clones and arrangements of eucalyptus in the north of Minas Gerais. Experimental randomized block with a split-plot design and five replications was used to evaluate sorghum. The effective productivity and height of sorghum comparing to the average values in the area were low. The GG 100 clone (*Eucalyptus urophylla* × *E. grandis*) showed low adaptation to semi-arid region while the MA 2001 clone (*Eucalyptus camaldulensis* × *E. Tereticornes*) presented greater height. The low values of some agronomic characteristics of sorghum were due to the distance between the rows of eucalyptus. The 2 × 3 + (15 m) and 2 × 3 + (20 m) arrangements of eucalyptus generated higher sorghum plants regardless of the clone.

Key words: Effective productivity, arrangements, height, sorghum, clone.

INTRODUCTION

The agroforestry system (SAF) is a conservation system that combines agricultural plants with tree species (Toma et al., 2013). Therefore, the agronomic features of the agricultural crops are influenced by the eucalyptus

*Corresponding author. E-mail: carlosjuliano@ufmg.br

component in the system.

Santos et al. (2015) mentioned in their work that intercropping corn and a forage crop with a tree component affects the growth and development of corn due to shading by trees and competition for nutrients between corn and the forage crop. The arboreal component in the SAF intercepts solar radiation, reducing the incident light in the under story which shades the crop. The low luminosity intensity can generate negative effects on sorghum relative to tillering increase, and finally, the reduction of biomass production (Clemente et al., 2015).

How to minimize the negative effects on culture and what is the best eucalyptus spatial arrangements to obtain the highest production yields? In this context, in addition to determine the best eucalyptus spatial arrangement, sorghum culture was used in agroforestry systems because it's has good capacity, power potential, and the essential features for the semi -arid as high yield and drought resistance (Santos et al., 2013). The forage crop was opted to grow because small producers in the north of Minas Gerais harvest it manually by cutting the panicles with machete for subsequent drying and storage with the remaining parts of the plant used as forage for the herd (Albuquerque et al., 2011). Therefore, the arrangements of trees can be manipulated through changes in density, row spacing, distribution of plants in line, and variability between plants (Argenta et al., 2001). There are numerous studies that show the influence of solar radiation on grasses grown in agroforestry systems. However, few studies have been done regarding sorghum crop, nevertheless, the crop yield has been promising in spatial arrangements of 2 × 3 + (20 m) and 2 x 10 m in the agroforestry systems of Minas Gerais-Brazil. (Clemente et al., 2015). This plant species has great ruggedness and acclimatization, and is grown all over Brazil (Coelho et al., 2002).

According to Clemente et al. (2015), sorghum productivity and height were higher in arrangements (2 × 3) + 20 m e 2 ×10 m, and evaluating sorghum locations in the plot, certified that the central side of the plants had higher yields. Gnanglè et al. (2013) evaluated the height and productivity of sorghum and cotton in and out of Shea cup (Butyrospermum Parkii paradoxa Gaertn) and obtained low yield and low plant height due light intensity decrease, indicating that plants closer to the trees have lower yields. The productivity of a crop depends on the density and spatial arrangement of the tree component, because during the second year after the deployment, the shading of trees interferes with crop yields (Neto et al., 2014). However, the use of broader arrangement, tree species with canopies that allow the passage of light to the understory, pruning and thinning techniques of eucalyptus may enable the use of annual crops longer in this system.

Given the above, the objective of this study was to evaluate the effect of the assessed sites on some agronomic characteristics such as height, grain yield and effective yield of BRS 655 forage sorghum intercropped with different clones of eucalyptus in different arrangements in semiarid SAF in Minas Gerais.

MATERIALS AND METHODS

The experiment was conducted in the municipality of Francisco Sá, a semiarid region of Minas Gerais, with tropical savanna climate (Aw), according to the Köppen climate classification, with an average annual rainfall of 981 mm and an average temperature of 22.3°C. The municipality is located at the coordinates of 16°07' S and 43°26' W, at an altitude of 591 meters with soil on the studied area classified as Atlantic Ultisol, according to EMBRAPA (System of Observation and Monitoring of Agriculture in Brazil).

Three eucalyptus clones were used: Clone 1 (MA 2001 – *E. camaldulensis* × *E. Tereticornes*); Clone 2 (A 144 – *E. urophylla x E. grandis*); Clone 3 (GG 100 – *E. urophylla* × *E. grandis*), in addition to BRS 655 sorghum hybrid. For the deployment of SAF, eucalyptus clones were planted in August 2012 with different arrangements in double rows: 2 × 3 m + (15 m) and 2 × 3 m + (20 m); triple rows 3 × 2 × 3 m + (10 m) and single rows 10 × 2 m. In August / 2012 held sub soiling in total area and were later made grooves for fertilization with reactive phosphate (330 g linear meter) and planting of eucalyptus clones in September / 2012. Sorghum seeds were planted between rows (alleys) of eucalyptus in November 2012 using a conventional planting method. During the second production season (2013) sorghum was sown again between rows of eucalyptus for the deployment of the experiment. The harvest was carried out in February after physiological maturity of grain.

The effect of structural arrangements of SAF with double rows: 2 × 3 m + (15 m) and 2 × 3 m + (20 m); triple rows 3 × 2 × 3 m + (20 m) and single rows 10 × 2 m. were studied. Sorghum crop was evaluated in between rows, that is, the larger spacing or alleys. The adopted spacing of sorghum BRS 655 was 0.80 m between lines and the initial population of plants was 140,000 ha^{-1}. Fertilization at planting was done using 400 kg ha^{-1} of a 04-30-10 (N-P-K) fertilizer. The distance of 1 meter from eucalyptus rows for planting sorghum was respected. The sorghum top dressing was performed when the plants had five fully expanded leaves and fully exhibited sheath. At this time, 300 kg ha^{-1} of a 20-00-20 (N-P-K) fertilizer was applied. During the preparation of the experimental area, a control of leaf-cutting ants was held by identifying the main nests in and around the area for later use of ant baits based on fipronil.

Thus, for each arrangement and clone an experiment with randomized blocks in split-plot design with five replications was implanted. The sorghum experimental plot consisted of 6 lines, each 4 m long, and subplots with 3 assessment areas of agronomic characteristics. To delimit the useful area of each arrangement, two central lines of sorghum planted between the rows of eucalyptus were considered, in addition to two north sidelines and two south sidelines of the forest component. On both sides there was a border line with sorghum in the direction of eucalyptus. The panicles were covered with paper bags at flowering for protection against birds.

During the experiment period, pesticide applications to control insects, diseases and weeds took place according to recommendations and practices commonly adopted in the region. The following agronomic sorghum evaluations were performed:

- Plant height (m): the plant height was taken from the insertion of the upper panicle to the ground, by measuring, in meters, four plants per plot after physiological maturity of grain.
- Grain yield (kg ha^{-1}): data of grain yield from the plots after threshing were corrected for moisture of 13% and transformed to kg ha^{-1} using the following Equation 1:

P13% = [PC(1-U)/0,87] (Clemente et al., 2015) (1)

Where:
P13%: grain yield (kg ha^{-1}) corrected to a standard humidity of 13%;
PC: grain yield without correction;
U: grain humidity during harvest.

Effective grain yield per arrangement was calculated using the per hectare grain yield, which is based on sorghum productivity of the system discounting the area occupied by trees. It was determined by the following equation 2:

Effective productivity (kg ha^{-1}) = {Productivity (kg ha^{-1}) x [(10000 – area occupied by trees (m^2)) / 10000)]}. (Clemente et al., 2015) (2)

Evaluations regarding development of eucalyptus were carried out after the harvest of sorghum. The circumference at breast height of all trees found in the area was measured on each parcel using a tape measure. The total height of the plants was determined with the aid of a digital hypsometer (MODEL Vertex III). Total height of all individuals was measured on each plot. The total average height on each plot was calculated using the arithmetic average of the individual heights on the plot.

The zenith angle and azimuth were calculated to evaluate the effect of eucalyptus shadow projection on the sorghum crop at 7:00 a.m., 9:00 a.m. and 15:00 on the 15^{th} day of each month.

The data obtained for sorghum crop were submitted initially to an individual analysis of variance per experiment (clone). At first, the tests of additivity of data, normality of errors and homogeneity of variances were performed. Later, analysis of variance was performed involving the three clones in different arrangements.

All analyzes were performed using the SISVAR® statistical program (Ferreira, 2000). The means were grouped by the Scott-Knott test at 5% significance.

RESULTS AND DISCUSSION

Tree mortality of all the studied clones was observed. It was found that the mortality rate was greater for clones of A144 and GG100, respectively in all arrangements (Table 1). The eucalyptus clone called MA 2001, performs better in semi-arid conditions due to increased tolerance to drought. The species of *E. camaldulenses,* used as a basis for the crossing and obtaining the MA 2001 clone, use water more efficiently (Zahid et al., 2010). This makes clear the greater height of the clone MA 2001 in the experiment. The GG 100 clone had lower plant height (AP) and smaller circumference at breast height (CAP), except the treatment of 2 × 3 × 2 + (10 m), due to lower tolerance to drought. The growth of eucalyptus was harmed probably by the lack of readily available water which caused a lower height.

The average height of eucalyptus in different arrangements was 7.48 m after 24 months. According to Peng et al. (2009), the tree height creates striking effects on crop. The greater the plant height is, the greater the competition for water, light and nutrients between crops. However, the competition may decrease when the distance between the rows of trees is larger. According to the author, the spacing between 15 to 20 m makes it a much more valuable route for the use of the culture in agroforestry system. By analyzing the A144 clone, there were no significant differences between the evaluation sites as a function of the arrangements: 2 × 3 m + (15 m), 2 × 3 m + (20 m), 2 × 10 m and 2 × 3 × 2 m + (10 m)

The GG100 clone did not reveal significant differences between the locals of assessment and arrangements of 2 × 3 m + (15 m) m and 2 × 3 × 2 m + (10 m), however, the other arrangements on the north side and center produced higher plants. This is partly because the GG 100 clone average height was 6.14 m, which less influenced the interception of solar radiation when comparing to MA 2001 with an average height of 9.06 m, having higher plants in central and north side. During sowing of sorghum the average eucalyptus height, regardless of clones, was 7.48 m after 24 months. Thus, it is important to know the range of average eucalyptus shadow to deploy planning in agroforestry system, considering that the choice of spacing between the rows of eucalyptus and the crop becomes a factor of production for better absorption of solar radiation in months after sowing, as well as dry matter accumulation and production of sorghum grain. The interaction between eucalyptus and sorghum possibly occurred due to mortality of eucalyptus plants near the sorghum plots triggering less competition between the plants

According to Clemente et al. (2015) the amount of solar radiation reaching among the eucalyptus ranks is crucial to the growth of sorghum in agroforestry system. The amount of light reaching may influence on the agronomic characteristics of the crop. So, the sorghum that is closer to the ranks of eucalyptus has lower height. According to the author, the central plants had a higher height, and this generates higher photosynthetic rate and redistribution of assimilates.

The same was observed by Macedo et al. (2006), who found plant height and weight of corn cob values above the average when the distance between the rows of eucalyptus was between 4.5 to 5.4 m. The average plant height, average weight of corn cob and grain yield were lower for the distances of 1.8 to 2.7 m, possibly because there was influence of incident light in the middle of alleys of eucalyptus clones, oriented east-west. There was a significant effect of all treatments on plant height. Regarding grain yield and effective grain yield, only the interaction of arrangement × assessed site provided no effect on agronomic evaluations (Table 2). These finding were agreed with Clemente et al. (2015) and Gnanglè et al. (2013).

The height of the BRS 655 sorghum hybrid can reach 2.50 m (Rodrigues et al., 2008), which demonstrates that the average height of 1.10 m found in the experiment was influenced by the shading of the culture. The sorghum plant possesses C4 metabolism, therefore, it needs high solar radiation to express its productive potential. The lack of easily available water in soil was also a factor that affected the physiology of sorghum regarding growth. The MA 2001 clone affected the heights of the plants

Table 1. Arithmetic mean of the number of plants after planting, final plans, index of mortality (%), plant height (AP), and breast height (CAP) of eucalyptus clones in different arrangements.

Clone	Arrangement	Initial plants	Final plants	Index of mortality (%)	AP (m)	CAP (m)
MA2001	2 x 3 +(15 m)	555	552.23	0.5	9.02	29.43
	2 x 3 +(20 m)	434	431.83	0.5	9.15	29.00
	2 x10 m	500	497.50	0.5	9.01	30.62
	2 x 3 x 2 + (10 m)	938	933.31	0.5	9.06	30.88
A144	2 x 3 + (15 m)	555	457.88	17.5	7.15	29.13
	2 x 3 + (20 m)	434	358.05	17.5	7.26	30.00
	2 x10m	500	412.50	17.5	7.23	21.00
	2 x 3 x 2+(10 m)	938	773.85	17.5	7.33	20.00
GG100	2 x 3 + (15 m)	555	480.63	13.4	6.16	21.13
	2 x 3 + (20m)	434	375.84	13.4	6.01	16.7
	2 x 10 m	500	433.00	13.4	6.27	18.31
	2 x 3 x 2 + (10 m)	938	812.31	13.4	6.11	22.32

Table 2. Summary of the analysis of variance of plant height (AP), grain yield (PG), and effective productivity (PGef) of sorghum grown between the different rows of eucalyptus in semiarid region.

Variation sources	Gl (df)	Mean square		
		AP (m)	PG (kg ha^{-1})	PGef (kg ha^{-1})
Bloc	5	0.04	1314421.70	750979.43
Arrangement (A)	3	1.57**	45470584.57**	54695479.56**
Clone (C)	2	1.45**	47782270.32**	19418343.63**
Local (L)	2	0.33**	48563603.38**	19912421.55**
A X C	6	0.22**	11174001.20**	5002243.41**
A X L	6	0.32**	3102229.64	1582974.83
C X L	4	0.13*	6676650.29*	2741152.93*
C X L X A	12	0.14**	8358075.84**	3818320.37**
Error	823	0.05	2318286.70	977191.37
Corrected Total	863			
CV (%)		19.72	36.09	36.2
Overall average		1.10	2303.69	1493.09

**: Significant at 1% of error probability by F test. *: Significant at 5% of error probability by F test.

depending on the place of evaluation and arrangement (Table 3). When evaluation was performed in the center, higher plants were observed in most arrangements. However, the assessment in the center did not differ statistically from the assessment carried out on the north side in the 2 × 3 m + (15 m) arrangement. In addition, the north side provided higher yield except of the treatment of 2 × 3 × 2 m + (10 m) arrangement (Table 3).

Regarding the arrangements within each assessment site, taller plants were observed for the MA 2001 clone in the 2 × 3 m + (20 m) arrangement measured on the south side and in the center, while north side produced higher plants observed in the 2 × 3 m + (15 m) arrangement (Table 3). The south side with the 144 clone produced higher plants in the 2 × 3 m + (15 m) and 2 × 3 m + (20 m) arrangements, in the center the 2 × 3 × 2 m + (10 m) arrangement produced lower plants with this clone (Table 3).

Regarding the third clone (GG100) the center and north side showed similar behavior having higher plants in the 2 × 3 m + (15 m) and 2 × 3 m + (20 m) arrangements, unlike the north side where plants had the same height (Tables 3 and 4). Importantly, in most places of evaluation on the plots, the 2 × 3 + (15 m) and 2 × 3 m + (20 m) arrangements showed higher plants regardless of the clone (Table 3). The sorghum crop evaluated on the

Table 3. Test of average plant height (m) of sorghum depending on the clones, local of assessment and arrangements with eucalyptus.

Clone	Local of assessment	Plant height (m)			
		Arrangements			
		2x3m+(15 m)	2x3m+(20 m)	2x10 m	2x3x2m+(10 \m)
MA2001	South	1.10^{bB}	1.24^{aA}	1.02^{bB}	1.13^{aB}
MA2001	Center	1.38^{aA}	1.09^{aB}	1.05^{bB}	1.19^{aB}
MA2001	North	1.33^{aA}	0.96^{cB}	1.18^{aB}	0.99^{bB}
A144	South	1.19^{aA}	1.25^{aA}	1.04^{aB}	1.06^{aB}
A144	Center	1.24^{aA}	1.30^{aA}	1.14^{aA}	1.06^{aB}
A144	North	1.39^{aA}	1.19^{aB}	1.09^{aB}	0.95^{aB}
GG100	South	0.98^{bA}	1.00^{bA}	1.01^{aA}	0.91^{aA}
GG100	Center	1.11^{aA}	1.17^{aA}	0.96^{aB}	0.97^{aB}
GG100	North	1.11^{aA}	1.13^{aA}	1.00^{aB}	0.88^{aB}

Means with the same lower case letter vertically within each clone arrangement belong to the same group, according to the Scott-Knott test. Horizontally, means with the same capital letter do not differ by F test at 5% probability.

Table 4. Eucalyptus shadow projection, width of shadow situated between eucalyptus and sorghum plants (D) and average shadow width (Average D).

Month	Shadow projection (m)			D (m)			Average D (m)
	7 o'clock	9 o'clock	15 o'clock	7 o'clock	9 o'clock	15 o'clock	Day (15)
November	21.64	6.94	6.94	2.36	4.12	4.12	3.53
December	20.80	6.93	6.93	3.59	3.55	3.55	3.56
January	21.26	6.93	6.93	2.90	3.88	3.88	3.55
February	23.39	7.12	7.12	0.17	4.93	4.93	3.35
March	27.76	7.84	7.84	4.28	6.16	6.16	5.54
April	37.06	9.41	9.41	12.29	7.83	7.83	9.32
May	51.20	11.31	11.31	23.46	9.60	9.60	14.22
Average							6.2

south side with the MA 2001 clone in the 2 × 3 + (20) arrangement had higher plants, probably due to greater spacing between the rows of eucalyptus - 20 m, which provided higher incidence of radiation (Table 3).

The south side with the 144 clone presented higher plants because of higher mortality of the trees in the experiment, which contributed to increased sunlight and less competition between crops. (Table 3). The evaluations in the center showed higher sorghum plants in most arrangements. This may have happened due to lower competition with eucalyptus for water and nutrients from soil or even due to passing diffused light enabling plant photosystems (Table 4). Sowing of sorghum took place during the summer solstice, when the direction of the Sun was east-southeast – west-northwest according the 83,75º azimuth. Over the months was observed the average width of the shadow of 6.2 m as shown in Table 4. Thus, the nearest sorghum lines to eucalyptus rows were shaded more. The north side of sorghum parcel was less affected by shading because the shadow was projected in the same direction of the Sun; however, the opposite side of the parcel was affected by the shadow.

Considering the distance of eucalyptus from sorghum of 1.0 m, width of the parcel of 4.8 m and the length of the shadow of 3.53 m, the two lines of the north parcel of sorghum were not affected by shadowing. Still, the center lines did not show significant difference from the north lines based on height and sorghum productivity. This was probably because of the scattered light passing through the eucalyptus canopy. By analyzing the results of calculated grain yield of sorghum (kg ha^{-1}), regarding different clones, locals of evaluation and eucalyptus arrangements we found with the MA 2001 clone that the assessment site had an effect in the 2 × 10 m and 2 × 3 × 2 m + (10 m) arrangements (Table 5). In this case, lower values were observed when the plots were evaluated on the south and north side of the 2 x 10 m single-row arrangement, and on the north side of the 2 × 3 × 2 m + (10 m) arrangement with triple rows. For the arrangements of 2 × 3 m + (15 m) and 2 × 3 m + (20 m) the evaluation sites did not affect grain yield.

Regarding the A144 clone, we found in the 2 × 3 m +

Table 5. Average test for calculated grain yield of sorghum (kg ha^{-1}), depending on the clones, local of evaluation and arrangements with eucalyptus.

Clone	Local of evaluation	Arrangements			
		2x3+(15 m)	2x3+(20 m)	2 x10 m	2 x 3 x 2+(10 m)
MA2001	South	2670.73^{aA}	2285.32^{aA}	1595.85^{bB}	1405.18^{bB}
MA2001	Center	3434.92^{aA}	1986.66^{aB}	3785.09^{aA}	3009.71^{aA}
MA2001	North	2905.29^{aA}	1385.06^{aB}	1461.12^{bB}	2449.30^{aA}
A144	South	3526.45^{aA}	2731.81^{bB}	2615.48^{bB}	2089.93^{aB}
A144	Center	3063.22^{aB}	3756.91^{aA}	3573.33^{aA}	2144.09^{aB}
A144	North	2685.25^{aA}	2621.08^{bA}	1676.50^{cB}	1642.14^{aB}
GG100	South	2719.72^{aA}	1732.28^{aB}	1497.16^{aB}	1005.28^{aB}
GG100	Center	2828.30^{aA}	2814.17^{aA}	1025.78^{aB}	1790.13^{aB}
GG100	North	2163.62^{aA}	2386.91^{aA}	1630.95^{aA}	838.27^{aB}

Means with the same lower case letter vertically within each clone arrangement belong to the same group, according to the Scott-Knott test. Horizontally, means with the same capital letter do not differ by F test at 5% probability.

Table 6. Average test for the effective productivity of grain of sorghum (kg ha^{-1}), as a function of clones, local of evaluation and arrangements of eucalyptus.

Clone	Local of evaluation	Arrangements			
		2x3+(15 m)	2x3+(20 m)	2x10 m	2x3x2+(10 m)
MA2001	South	1781.38^{aA}	1690.22^{aA}	1117.09^{bB}	614.35^{bB}
MA2001	Center	2291.09^{aA}	1469.34^{aB}	2649.55^{aA}	1315.85^{aB}
MA2001	North	1937.83^{aA}	1024.39^{aB}	1022.78^{bB}	1070.83^{aB}
A144	South	2352.14^{aA}	2020.45^{bA}	1830.84^{bA}	913.72^{aB}
A144	Center	2043.17^{aB}	2778.61^{aA}	2501.33^{aA}	937.39^{aC}
A144	North	1791.06^{aA}	1938.55^{aA}	1173.55^{cB}	717.94^{aB}
GG100	South	1814.06^{aA}	1281.20^{bB}	1048.01^{aB}	439.51^{aB}
GG100	Center	1886.47^{aA}	2081.36^{aA}	718.04^{aB}	782.65^{aB}
GG100	North	1443.13^{aA}	1765.36^{aA}	1141.66^{aA}	366.49^{aB}

Means with the same lower case letter vertically within each clone arrangement belong to the same group, according to the Scott-Knott test. Horizontally, means with the same capital letter do not differ by F test at 5% probability.

(20 m) and 2 × 10 m arrangements that sorghum yields were reduced approaching the trees with central portion having higher yields. In other arrangements there was no effect of local assessment on productivity of sorghum (Table 4). Table 5 also shows that the grain yield on the south side with the MA 2001 clone was higher in the 2 × 3 m + (15 m) and 2 × 3 m + (20 m) arrangements; in the center in the 2 × 3 m + (15 m), 2 × 10 m and 2 × 3 × 2 m + (10 m) arrangements; and north side in the 2 × 3 m + (15 m) and 2 × 3 × 2 m + (10 m) arrangements. Regarding the A144 clone, the south side of the 2 × 3 m + (15 m) arrangement; the center in the 2 × 3 m + (20 m) and 2 × 10 m and the north side in 2 × 3 + (15 m) arrangements produced higher yield. Finally, the GG 100 clone produced higher yields on the south side in the 2 × 3 m + (15 m) arrangement; center in the 2 × 3 m + (15 m) and 2 × 3 m + (20 m) arrangements; and north in the 2 × 10 arrangement. The effective grain yield with different clones according to the locations of evaluation and arrangements are shown in Table 6. All clones, regardless of the site of assessment, in the 2 × 3 + × 2 m (10 m) arrangement showed the lowest effective productivity, as it was expected because of bigger number of trees.

According to the Ministry of Agriculture (Brasil, 2015), the average productivity of sorghum in the northern region of Minas Gerais reaches 1891 kg ha^{-1}. The average effective yield of sorghum in agroforestry system was below when compared to the average productivity in this region. The competition for water, nutrients and light most likely caused low productivity of the crop. However, the effective productivity of sorghum in the 2 × 3 + (15 m) and 2 × 3 + (20 m) arrangements generated high and productive plants (Tables 5 and 6). When comparing the evaluation sites in each arrangement, the same trend of grain yield was observed for effective grain yield. It shows that the sorghum plants which are closer to the clones have smaller effective yield. Therefore, it is recommended that plots involving sorghum intercropped with eucalyptus are well planned for better representation of the environment.

Conclusion

The planning of deployment of sorghum crop in agroforestry system must be made considering the average range of eucalyptus shadow, a greater spacing between the rows of trees and their distance to the crop. The height and effective yield of sorghum were higher in central and north lines of the plot. The 2 × 3 + (15 m) and 2 × + 3 (20 m) arrangements of eucalyptus generated higher sorghum plants regardless of the clone.

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGMENTS

The authors thank FAPEMIG, CNPq and CAPES for their financial support.

REFERENCES

Albuquerque CJB, Von Pinho RG, Rodrigues JAS; Brant RS (2011). Row spacing and sowing density of forage sorghum in the northern region of Minas Gerais. Ciênc. Agrotec. 35(3):494-501.

Argenta G, Silva PRF, Sangoi L (2001). Maize plant arrangement: analysis of the state of the art. Ciênc. Rural Santa Maria 31(6):1075-1084.

Brasil (2015). Sorgo. Subsecretaria do Agronegócio, Belo Horizonte. Available at: <http://www.agricultura.mg.gov.br/images/Arq_Relatorios/Agricultura/2015/jun/perfil_sorgo_jun_2015.pdf>. Accessed on: December,10,2015.

Clemente MA, Zandondi CHS, Lana RMQ, Franco FO, Albuquerque CJB (2015). Agricultural characteristics and evaluation locations of sorghum plots with different eucalyptus arrangements. Afr. J. Agric. Res. 10(32):3093-3100.

Coelho MA, Waquil JM, Karam D, Casela CR, Ribas, PM (2002). Seja doutor do seu sorgo. Potafós, (Arquivo do agrônomo, 14), Piracicaba. Available at: http://brasil.ipni.net/article/BRS-3150

Gnanglè CP, Gbemavo C, Aïhou K, Kakaï RG, Sokpon N (2013). Productivity of cotton and sorghum in an agroforestry system of shea trees (*Vitellaria paradoxa gaertn*) in northern Benin. Nat. Sci. 5(2): 207-213.

Macedo RLG, Bezerra RG, Venturin N, Vale RS, Oliveira, TK (2006). Silvicultural performance of eucalyptus clones and agronomic characteristic of corn planted in agroforestry systems. Rev. Árvore Viçosa 30(5):701-709.

Neto MMG, Viana MCM, Alvarenga RC, Santos EA, Simão EP, Campanha MM (2014). Crop-livestock-forest integration systems in Minas Gerais. B. Ind. Anim. Nova Odessa 71(2):183-191.

Peng X, Zhang Y, Cai J (2009). Photosynthesis, growth and yield of soybean and maize in a tree-based agroforestry intercropping system on the Loess Plateau. Agrofor. Syst. 76:569-577.

Rodrigues JAS, Santos FG, Ferreira AS, Casela CR, Tardin FD (2008). BRS 655 - Híbrido de sorgo forrageiro para produção de silagem de alta qualidade. Circular Técnica. Embrapa, Sete Lagoas-MG, Dezembro, 2008. Available at: http://www.cnpms.embrapa.br/publicacoes/publica/2008/circular/Circ_107.pdf

Santos RD, Pereira LGR, Neves ALA, Rodrigues JAS, Costa, CTF, Oliveira, GF (2013). Agronomic characteristics of forage sorghum cultivars for silage production in the lower middle San Francisco Valley. Acta Scientiarum, Animal Sciences. Maringá 35(1):13-19.

Santos MV, Silva DV, Fonseca DM, Reis MR, Ferreira LR, Neto SNO, Oliveira FLR (2015). Productive components of maize under different weed management systems and planting arrangements in agrosilvopasture system; Ciênc. Rural, Santa Maria 45(9):1545-1550.

Toma RS, Cooper M, Tavares MF, Carvalho CC, Teato AC (2013). Temporal evolution of soil hydro-physical behavior of an agroforestry system on Ribeira Valley – SP. Sci. For. Piracicaba 41(98):237-248.

Zahid DM, Shah FUR, Majeed A (2010). Planting eucalyptus camaldulensis in arid Environment - is it useful species under Water deficit system?. Pak. J. Bot. 42(3):1733-1744.

academicJournals

Vol. 11(33), pp. 3111-3122, 18 August, 2016
DOI: 10.5897/AJAR2016.11121
Article Number: 62B549B60042
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Geographic patterns of phenotypic diversity in sorghum (*Sorghum bicolor* (L.) *Moench*) landraces from North Eastern Ethiopia

Haile Desmae[1,4]*, David R. Jordan[2] and Ian D. Godwin[1,3]

[1]School of Agriculture and Food Sciences, the University of Queensland, QLD, 4072, Australia.
[2]Queensland Alliance for Agriculture and Food Innovation, the University of Queensland, Warwick, QLD, 4370, Australia.
[3]Alliance for Agriculture and Food Innovation (QAAFI), the University of Queensland, St Lucia, QLD, 4072, Australia.
[4]ICRISAT-WCA, BP 320, Bamako, Mali.

Received 12 April, 2016; Accepted 8 July, 2016

Understanding the pattern of genetic variability is an important component of germplasm collection and conservation as well as the crop's improvement process including the selection of parents for making new genetic recombination. Nine hundred seventy four sorghum landraces from North Eastern (NE) Ethiopia were evaluated for agro-morphologic characters to assess geographic patterns of phenotypic diversity and to identify whether there are specific areas of high diversity for particular traits. The Shannon-Weaver diversity index (*H'*) for qualitative traits ranged from 0.30 to 0.93 (mean = 0.67) for grain covering and grain color, respectively. The landraces also displayed highly significant differences (p<0.01) for all the quantitative traits with days to flowering ranging from 64 to 157 days (range = 93), days to maturity from 118 to 215 (range=97) days, plant height from 115 to 478 cm; 1000-seeds weight from 18 to 73 g, and grain number from 362 to 9623. The first five principal component axes captured 71% of the total variation with days to flowering and maturity, leaf number and length, panicle weight, grain weight and number per panicle, panicle length, length of primary branches, 1000-seeds weight and internode length accounting for most of the variability. Cluster analysis grouped the landraces into ten clusters. The clustering of zones and districts revealed close relationship between geographic locations based on proximities and agro-ecological similarities. Differentiation analysis showed that most of the landraces variability was within rather than between geographic origins of the landraces, indicating weak genetic differentiation among landraces from predefined geographic origins such as political administrative zones and districts. The weak differentiation might be due to frequent gene flow across the study area because of seed exchanges among farmers.

Key words: Differentiation, diversity, landraces, traits, zones.

INTRODUCTION

Sorghum is an African domesticate, particularly in the Ethiopia/Sudan region of Eastern Africa (Vavilov, 1951; Harlan and de Wet, 1972; Stemler et al., 1977). Ethiopia is a center of diversity for sorghum (Gebrekidan, 1973; Doggett, 1988) with four of the main five races of sorghum and their corresponding subraces cultivated (Doggett, 1988; Teshome et al., 1997; Ayana and Bekele, 1998). Various germplasm collection missions resulted in large number of accessions conserved in national and international conservation centers as well as breeding institutions (Gebrekidan, 1982; Prasada Rao and Mengesha, 1987). The collections showed useful variability for various traits such as grain quality, disease resistance, drought resistance and other desirable agronomic characteristics (Singh and Axtell, 1973; Prasada Rao and Mengesha, 1987; Kebede, 1991; Tegegne et al., 1994; Tuintsra et al., 1997; Subudhi et al., 2000; Wu et al., 2006; Reddy et al., 2006; Kassahun et al., 2010).

North-eastern (NE) Ethiopia is one of the main sorghum growing areas of the country with the crop ranking second next to tef (*Eragrostis tef*) in area sown and first in production (CSA, 2003). It is among the important areas for germplasm collection. The high lysine sorghum lines came from Welo collections (Singh and Axtell, 1973). The majority of sorghum production in the country in general and NE Ethiopia in particular depends on landraces (Gebrekidan, 1973; Worede, 1992; Seboka and van Hintum, 2006; Shewayrga et al., 2008). These landraces are good sources of genepool for sorghum improvement program to develop high yielding and farmer preferred improved varieties. Understanding the genetic variability available and its potential use in future breeding programs is important component of the crop improvement process including the selection of inbred parental materials for hybridization for making new genetic recombination. It also helps to devise appropriate sampling procedures for germplasm collection and conservation purpose including the establishment of a core collection with maximum genetic diversity (Brown, 1995; Kresovich et al., 1995; Hayward and Sackville-Hamilton, 1997; Ramanatha Rao and Hodkgin, 2002; Kahilainen et al., 2014; Govindaraj et al., 2015).

Analysis of morphological diversity is one of the important and useful techniques employed to determine variability in different crops (Assefa and Labuschagne, 2004; Haussmann et al., 1999; Grenier et al., 2004; Gerrano et al., 2014; Dossou-Aminon et al., 2015; Mengistu et al., 2015). In the case of sorghum in Ethiopia, Gebeyehu (1993) recorded significant difference in quantitative traits among 59 landraces from Gambella in Western Ethiopia. McGuire et al. (2002) observed high phenotypic diversity in sorghum landraces from two districts (Meiso and Chiro) in Eastern Ethiopia, where variation among farmers in their ecological conditions and needs contributes to overall varietal diversity. At a wider scale, Ayana and Bekele (1998) reported high morphological diversity among 347 Ethiopian sorghum accessions for qualitative and quantitative traits. The studies on sorghum landraces from NE Ethiopia were limited in terms of the nature of sampling and number of samples considered, and the number of sites sampled and the coverage of sorghum growing areas. Either small samples of accessions from gene banks (Ayana and Bekele, 1998) or collections from few targeted sites (Abdi et al., 2002) were included in the studies. The objectives of the present study were to investigate the extent and geographic pattern of phenotypic diversity in sorghum landraces in terms of districts, zones and altitude classes of NE Ethiopia using large sample of representative landraces, and to identify whether there are specific areas of high diversity for particular traits.

MATERIALS AND METHODS

A total of 974 landraces that included 307 from North Welo, 363 from South Welo, 129 from Oromiya and 175 from North Shewa administrative zones were evaluated covering more than 350 km distance of north to south stretch (Figure 1). A total of 14 sorghum growing districts were covered in the study that included Kobo, Gubalafto, Habru and Meket from North Welo; Ambassel, Tehuledere, Dessie Zuria, Kalu, and Debresina from South Welo; Bati and Artuma Jille from Oromiya; and Kewot, Tegulet and Merabete from North Shewa. Some districts used in the analysis represent two or more of the current administrative delineation as most of the passport data available from the Institute of Biodiversity Conservation (IBC) relate to old classification. For example, Merabete represents all the present districts in that boundary; Kewot represents Kewot and Efratana Gidim; and Artuma Jille represents both Artuma Fursina Jille and Chafa Gola (Dawa Chafa) districts. A classification was made to group landraces based on altitude as lowland (<1650 m), intermediate (1650-2000 m) and highland (>2000 m above sea level) areas. The landraces were tested at Sirinka (1850 m above sea level), North Welo, Ethiopia. Data were recorded for seventeen quantitative and eight qualitative traits using sorghum descriptors (IBPGR/ICRISAT, 1993). The data for quantitative traits were averages from five randomly selected plants while the data for qualitative traits were recorded at plot level.

Data analysis

For qualitative traits, phenotypic frequency distributions and Shannon-Weaver diversity index (H′) were estimated for all the landraces, districts, altitude and zones. The Shannon-Weaver diversity index (Ayana and Bekele, 1998) for a trait is given as: $H' = -\sum P_i \log_e P_i$, where, P_i is the proportion of landraces in the i[th] class of an n-class trait (the number of phenotypic classes of a trait).

The variability for quantitative traits was described using mean, range and analysis of variance (ANOVA) with randomized complete block design. Cluster analysis was performed based on the quantitative and qualitative traits with Gower's generalized distance estimates and ward clustering method to group the landraces using

*Corresponding author. E-mail: hailshd@yahoo.com.au, h.desmae@cgiar.org. Tel: +223-71178098.

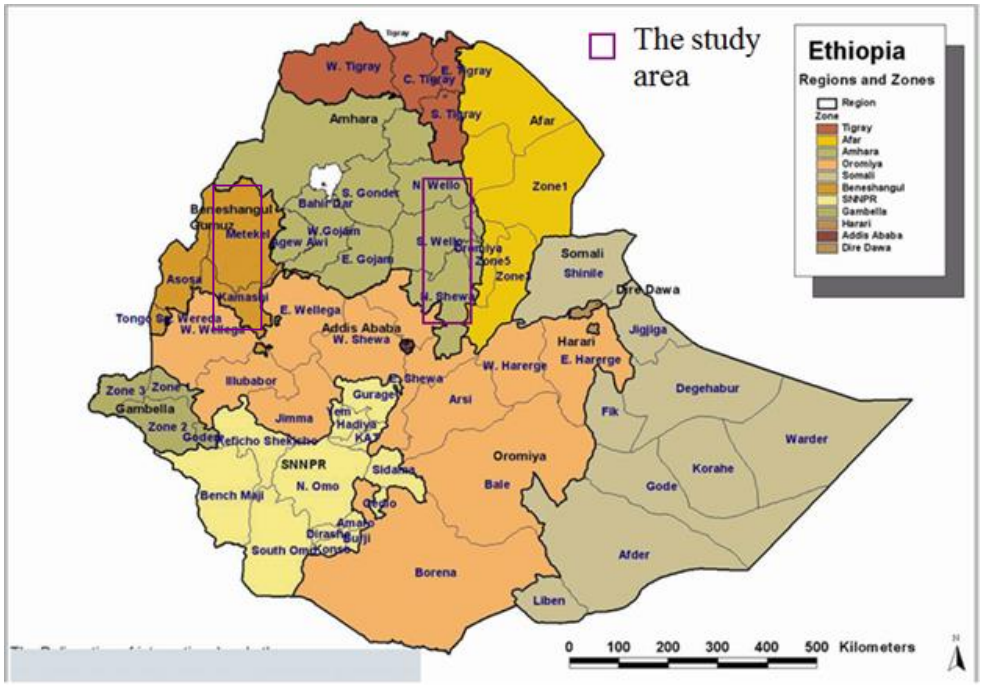

Figure 1. Map of Ethiopia and the study area.

daisy in R-Project Software Package (R core team, 2010). For the cluster analysis, some 21 landraces from Harerge (Eastern Ethiopia) and 9 improved varieties were included for comparison purpose. Cluster analysis was also performed for districts and zones using mean for quantitative traits. Principal component analysis (PCA) was performed after standardizing the quantitative data.

RESULTS

Variability for qualitative traits

A high variability for qualitative traits was observed in the landraces. The distribution of phenotypic classes for the entire NE Ethiopia showed the predominance of particular trait classes in the landraces for the qualitative traits. Non-juicy types (92%), awns at maturity (67%), white midrib color (72%), grey and straw glume color (63%), semi-compact to compact head types (68%), mostly starchy and completely starchy (82%), and 25% grain covered (90%) characterize the majority of landraces (Supplementary Table 1). White, red and light red, straw, yellow, and brown seed color were important accounting for more than 81% of the landraces. A similar trend was observed in the distribution of the trait classes in the four administrative zones and three altitude ranges, but localities of specific diversity for trait classes were observed. For example, the proportion of red brown seed color, a predominant color of Zengada, was higher in South Welo as compared to the other three zones. Red seed color was not common in North Shewa relative to other zones. The proportion of grey seed color was relatively high in North Welo and Oromiya. These are landraces such as Tikureta (e.g. Gubete, Wanose, leza), Mogayfere and Homdade, which are mainly grown for roasted grain (‘eshet’) or local beverages. The proportion of black and white glume colors was relatively higher in Oromiya reaching 19 and 14%, respectively, which was low in the other zones.

In the case of altitudes, awns at maturity, panicle compactness, grain color and endosperm texture showed some clinal variation. Presence of awns at maturity was a frequent trait ranging from 64% of landraces for the lowland and intermediate altitude to 82% for the highland areas. Semi-compact elliptic and compact elliptic head types were more frequent in the lowland and intermediate altitude areas accounting for 67 and 69% of the landraces, respectively. In higher altitude areas, semi-open head types were equally important as the semi-compact elliptic types, while compact elliptic types were less frequent. The distribution of grain color showed a predominance of white, yellow, brown, straw, red and light red in the lowland and intermediate areas. In comparison, red brown followed by brown were the frequent grain colors in higher altitude areas. Mostly starchy endosperm types were more frequent in the lowland (55%) and intermediate areas (72%). In contrast, completely starchy endosperm and mostly starchy types were important in the highland areas accounting for 44 and 39% of the landraces, respectively.

Estimates of H′ revealed high qualitative traits diversity in the sorghum landraces of NE Ethiopia in general

Table 1. Shannon–Weaver diversity index (H′) of eight qualitative traits for sorghum landraces from NE Ethiopia by districts, zones and altitudes.

Geographic domains	Stalk juiciness - SJ	Leaf midrib color - LMC	Panicle compactness and shape - PCS	Awns at maturity - AM	Glume color - GlC	Grain covering - GCov	Grain color - GC	Endosperm texture -ET	Mean
NE Ethiopia	0.39	0.69	0.65	0.91	0.83	0.30	0.93	0.67	0.67±0.08
Zones									
North Welo	0.22	0.59	0.58	0.95	0.82	0.24	0.88	0.53	0.60±0.10
South Welo	0.50	0.77	0.67	0.78	0.79	0.28	0.94	0.63	0.67±0.07
Oromiya	0.50	0.64	0.67	0.99	0.86	0.29	0.92	0.75	0.70±0.08
North Shewa	0.19	0.63	0.65	0.95	0.82	0.41	0.89	0.66	0.65±0.09
Altitude									
≤1600	0.38	0.67	0.65	0.94	0.85	0.34	0.92	0.70	0.68±0.08
1601-2000	0.50	0.77	0.58	0.95	0.82	0.15	0.91	0.50	0.65±0.09
>2000	0.19	0.59	0.63	0.67	0.76	0.31	0.89	0.67	0.59±0.08
Districts									
Kobo	0.31	0.64	0.62	0.89	0.77	0.33	0.91	0.57	0.63±0.08
Gubalfto	0	0.52	0.52	0.97	0.80	0	0.78	0.50	0.52±0.12
Habru	0.31	0.59	0.49	0.97	0.82	0.17	0.90	0.45	0.59±0.10
Meket	0	0.22	0.68	0.57	0.81	0.18	0.74	0.63	0.48±0.11
Ambassel	0.52	0.71	0.56	0.75	0.73	0.43	0.93	0.56	0.65±0.06
Tehuledere	0.54	0.93	0.50	0.95	0.72	0.12	0.64	0.49	0.61±0.09
Dessie zuria	0	0.72	0.44	0	0.78	0.55	0.54	0.25	0.41±0.11
Kalu	0.57	0.80	0.68	0.83	0.71	0.29	0.91	0.60	0.67±0.07
Debre Sina	0	0.39	0.49	0.71	0.73	0.31	0.66	0.46	0.47±0.09
Bati	0.87	0.74	0.47	0.79	0.71	0.11	0.56	0.31	0.57±0.09
Artuma Jille	0.42	0.63	0.59	1.00	0.87	0.32	0.91	0.62	0.67±0.08
Shewa Robit	0.60	0.61	0.64	1.00	0.87	0.44	0.86	0.75	0.72±0.06
Tegulet	0.23	0.44	0.47	0.38	0.58	0.29	0.81	0.78	0.50±0.08
Merabete	0.19	0.73	0.44	0.72	0.62	0.35	0.82	0.63	0.56±0.08

and for each district, zone and altitude (Table 1). The diversity index across NE Ethiopia ranged from 0.30 to 0.93 (mean H′ = 0.67) for grain covering and grain color, respectively. The mean H′ across collection zones ranged from 0.60 for landraces in North Welo to 0.70 for landraces in Oromiya zone. The H′ estimates for the three-altitudes showed an increasing trend from highland to lowland for most of the traits with mean range of 0.59 to 0.68, respectively. The mean H′ at district level was a reflection of the diversity observed at the altitudinal level. Districts from higher altitude areas like Meket, Dessie Zuria, Debre Sina and Tegulet showed low diversity for many traits resulting in low mean

Table 2. Partitioning of the phenotypic variability into within and between zones of origin, altitudes and districts of NE Ethiopia using the method of Ayana and Bekele (1998).

Traits	Zones				Altitudes			Districts		
	H'_{NEE}	H'_Z	$\frac{H'_Z}{H'_{NEE}}$	$\frac{H'_{NEE}-H'_Z}{H'_{NEE}}$	H'_A	$\frac{H'_A}{H'_{NEE}}$	$\frac{H'_{NEE}-H'_A}{H'_{NEE}}$	H'_D	$\frac{H'_D}{H'_{NEE}}$	$\frac{H'_{NEE}-H'_D}{H'_{NEE}}$
SJ	0.39	0.35	0.90	0.10	0.36	0.91	0.09	0.33	0.84	0.16
LMC	0.69	0.66	0.95	0.05	0.68	0.98	0.02	0.62	0.90	0.10
PCS	0.65	0.64	0.99	0.01	0.62	0.96	0.04	0.54	0.84	0.16
AM	0.91	0.91	1.00	0.00	0.85	0.94	0.06	0.75	0.83	0.17
GlC	0.83	0.82	0.99	0.01	0.81	0.98	0.02	0.75	0.90	0.10
GCov	0.30	0.30	1.00	0.00	0.27	0.89	0.11	0.28	0.95	0.05
GC	0.93	0.91	0.98	0.02	0.91	0.98	0.02	0.78	0.84	0.16
ET	0.67	0.64	0.96	0.04	0.62	0.93	0.07	0.54	0.81	0.19
Mean	0.67	0.65	0.97	0.03	0.64	0.95	0.05	0.57	0.86	0.14

H'_{NEE} = Diversity index for the entire data of NE Ethiopia; H'_Z, H'_A and H'_D = mean diversity index for each trait for the four zones, three altitudes and fourteen districts, respectively; H'_Z/H'_{NEE}, H'_A/H'_{NEE} and H'_D/H'_{NEE} = proportion of diversity within zones, altitudes and districts, respectively; $(H'_{NEE} - H'_Z)/H'_{NEE}$, $(H'_{NEE} - H'_A)/H'_{NEE}$ and $(H'_{NEE} - H'_D)/H_{NEE}$ = proportion of diversity between zones, altitudes and districts, respectively, in relation to total variation. SJ= stalk juiciness, LMC=leaf midrib color, PCS=panicle compactness and shape, AM=awns at maturity, GlC=glume color, GCov=grain covering, GC=grain color, ET=endosperm texture.

diversity. The H′ value for grain color ranged from 0.54 to 0.93 for landraces from Dessie zuria and Ambassel, respectively. Landraces from Gubalafto, Dessie zuria, Meket, Debre Sina were all non- juicy types. Similarly, landraces from Dessie zuria were monomorphic for absence of awns at maturity. Overall, awns at maturity, grain color and glume color were the most diverse traits, while grain covering and stalk juiciness displayed the lowest diversity.

Partitioning of the qualitative traits variability into between and within zones of origin, altitudes and districts revealed that most of the variation was found to be within rather than between geographic origins of the landraces (Table 2). Ninety seven percent of the variation was within zones while only three percent was between zones. Stalk juiciness contributed relatively more (10%) to between zones differentiation. Similarly, the differentiation between altitudes appeared to be weak where 95% of the variation is accounted for by within altitude variation. Grain covering followed by stalk juiciness contributed to between altitude differentiation. Landraces between districts displayed relatively higher differentiation as compared to zones and altitudes, and almost all traits contributed to the differentiation.

Variability for quantitative traits

The landraces showed a wide range of variability for the quantitative traits (Table 3). Days to flowering ranged from 64 to 157 days, and days to maturity from 118 to 215 days. Plant height ranged from 115 to 478 cm; panicle weight from 21.8 to 443.4 g; grain weight per panicle from 11.87 to 348.23 g; 1000-seeds weight from 18 to 73 g; threshing percent from 29.6 to 93.6%; and grain number from 362 to 9623.

ANOVA for the entire NE Ethiopia data, zones and districts showed highly significant difference (P<0.01) for all traits among landraces (Table 4). Mean separation values for each trait are given in supplementary Table 2. Landraces from South Welo had higher values for panicle weight, grain weight per panicle and number of grain per panicle. Landraces from North Welo were relatively early flowering and maturing while landraces from North Shewa were late flowering and maturing. Similarly, highly significant differences were observed between altitudes for all traits except length of primary branches per panicle. Landraces from highland areas were late flowering and maturing with significantly low mean values for panicle weight, grain weight per panicle, 1000-seeds weight and number of grains per panicle as compared to lowland and intermediate altitude landraces. The variation between districts was highly significant for all the traits. Landraces from higher altitude districts like Tehuledere, Dessie zuria, Meket, Debre Sina and Tegulet were late flowering and maturing with low mean value for 1000-seeds weight. The variations among landraces were also highly significant within zones, districts and within altitudes.

Cluster analysis

The cluster analysis resulted in clustering of the landraces into ten groups, with minimum similarity level of 0.64 (Figure 2A). A total of 179 landraces were grouped in Cluster I, 89 in cluster II, 128 in cluster III, 198 landraces in cluster IV, 94 landraces in cluster V, 78

Table 3. Variability for quantitative traits described with minimum and maximum values, range and mean for the entire data.

Quantitative traits	Minimum	Maximum	Range	Mean (±SE)
Days to 50% flowering – DF	64	157	93	103±0.44
Days to 90% maturity – DM	118	215	97	168±0.54
Leaf number – LN	7	25	19	15±0.08
Leaf length(cm) – LL	54	113	59	88±0.33
Leaf width (cm) – LW	6	14	8	10±0.05
Internode length(cm) – IL	9	32	23	22±0.12
Leaf sheath length(cm) – LSL	9	39	30	20±0.08
Plant height(cm) – PH	115	478	363	299±1.89
Peduncle exertion(cm) – PE	0	34	34	5±0.16
Panicle length(cm) – PL	11	51	40	23±0.21
Number of primary branches per panicle - NPB	20	115	95	66±0.40
Length of primary branches(cm) - LPB	3	23	20	9±0.09
Panicle weight(g) – PW	22	443	422	143±1.92
Grain weight per panicle(g) – GW	12	348	336	112±1.59
1000 seeds weight(g) – SW	18	73	55	39±0.25
Threshing percent (%) – ThP	30	94	64	78±0.20
Grain number per panicle – GN	362	9623	9261	2858±35.26

Table 4. Mean square for variation between zones, districts and altitude groups, and between landraces for the entire data (within region) and within each zone from ANOVA for quantitative traits.

Trait	Between landraces of NE Ethiopia (df = 973)	Between geographic domains			Between landraces within zone			
		Zones (df = 3)	Districts (df = 13)	Altitudes (df = 2)	*North Welo* (df = 306)	*South Welo* (df = 362)	*Oromiya* (df = 128)	*North Shewa* (df = 174)
DF	379.4**	16933.7**	6145.9**	13864.7**	361.4**	306.4**	339.3**	305.9**
DM	567.3**	21086.8**	7790.1**	13515.9**	538.8**	490.6**	678.5**	341.5**
LN	13.1**	169.7**	79.8**	153.0**	14.6**	12.2**	13.5**	9.5**
LL	208.6**	2125.8**	732.6**	692.2**	245.6**	174.6**	220.4**	172.3**
LW	4.6*	69.8**	27.7**	32.1**	4.7**	4.3**	4.6**	4.1**
IL	27.5**	382.6**	160.4**	225.6**	29.1**	23.8**	28.8**	25.5**
LSL	12.5**	55.1*	42.4**	45.0**	10.2**	12.6**	12.0**	16.1**
PH	6787.2**	36091.2**	16671.7**	31312.5**	6455.0**	6455.0**	10530.5**	5878.0**
PE	53.6**	217.3**	132.5**	163.5**	67.6**	46.7**	42.5NS	48.8**
PL	92.2**	2308.6**	829.8**	2694.2**	79.8**	87.4**	64.6**	106.2**
NPB	269.8**	1245.1**	723.5**	481.6**	259.0**	252.3**	347.7**	251.0*
LPB	16.1**	127.6**	85.7**	351.8NS	13.9**	16.9**	18.3**	14.9**
PW	7468.3**	51626.0**	30007.39**	123361.7**	6481.0**	9296.8**	5854.0**	5826.2**
GW	5112.2**	35767.9**	22405.8**	84253.3**	4416.5**	6378.8**	4005.4**	3986.1**
SW	121.6**	919.5**	1145.4**	2677.8**	104.2**	136.7**	122.5**	106.4**
ThP	82.8**	1031.0**	498.4**	1389.8**	49.2**	88.0**	125.5**	83.5**
GN	2523400	29091510**	9559810**	18182778**	1855992**	3188461**	1910579**	2306225**

NS = Not significant; * significant at P<0.05; ** significant at P<0.01; DF=Days to 50% flowering, DM=Days to 90% maturity, LN=Leaf number, LL=Leaf length(cm) , LM=Leaf width (cm), IL= Internode length(cm) , LSL=Leaf sheath length(cm), PH=Plant height(cm), PE=Peduncle exertion(cm), PL=Panicle length(cm), NPB=Number of primary branches per panicle, LPB=Length of primary branches(cm), PW=Panicle weight(g), GW=Grain weight per panicle(g), SW=1000 seeds weight(g), ThP=Threshing percent (%), GN=Grain number per panicle

landraces in cluster VI, 61 in cluster VII, 52 in cluster VIII, 48 in cluster IX and 77 landraces in cluster X. The mean value of quantitative traits and frequency distribution ofqualitative traits for each cluster are given in Supplementary Tables 3 and 4, respectively. Cluster I included landraces characterized by above average

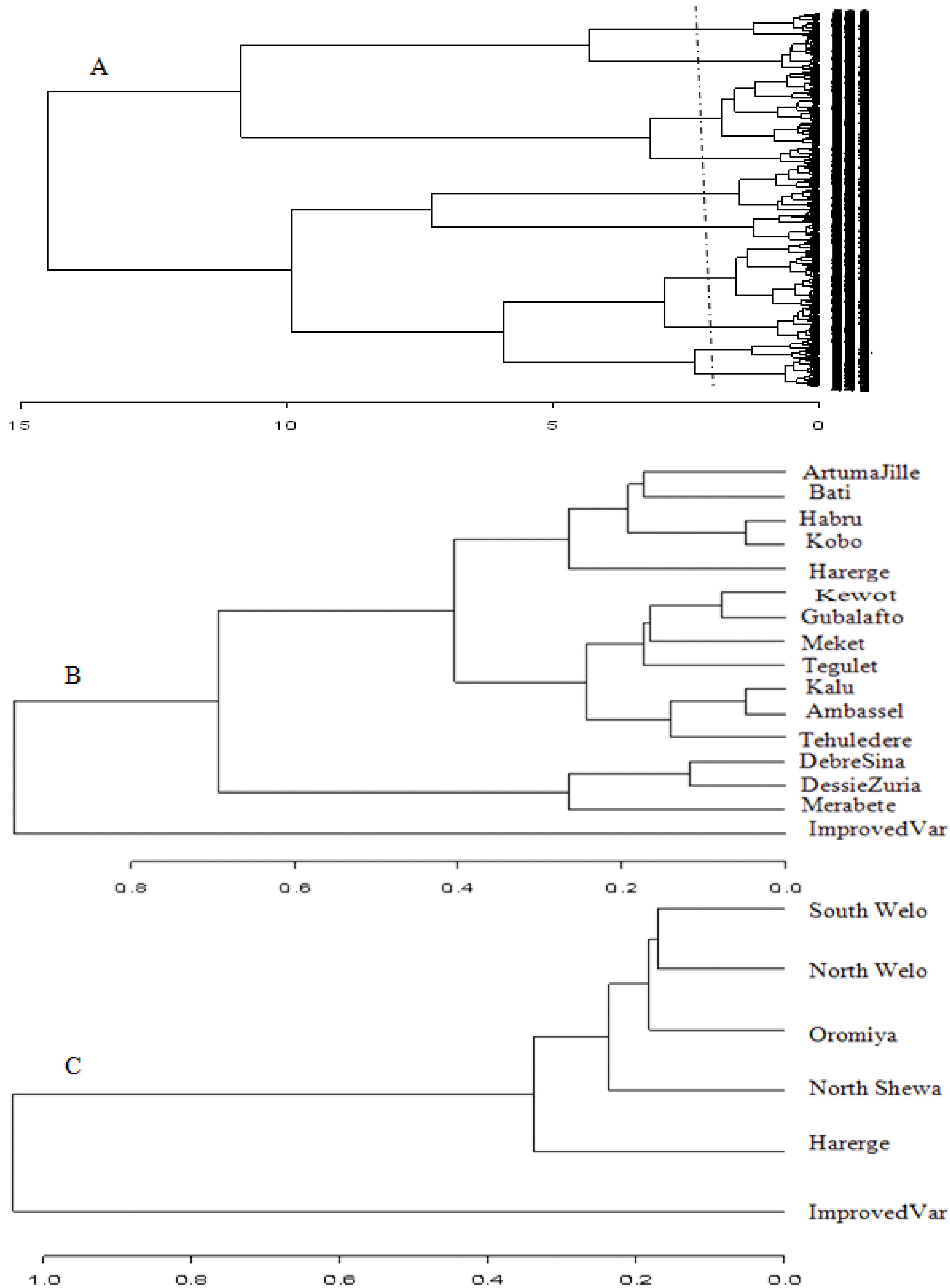

Figure 2. Dendrograms showing the clustering patterns for: A) landraces, B) districts and C) zones.

values for most quantitative traits. However, it was below average values for internode length, peduncle exertion, panicle length and length of primary branches. Straw and grey glume colors, white and yellow seed color and presence of awns at maturity were frequent traits. Semi-compact elliptic head types and mostly starchy endosperm types were predominant trait classes in the cluster. Cluster II contained landraces with above average value for all quantitative traits except peduncle exertion, with predominant qualitative traits of grey and straw seed color, straw glume color, mostly starchy endosperm and compact elliptic head type. Landraces such as Jamyo and Degalet types, which are preferred for various end-use qualities, were grouped in clusters I and II. Cluster III also showed above average values for all quantitative traits except panicle length and length of primary branches. Semi-compact elliptic followed by compact elliptic panicles, and grey and straw glume colors characterize most of the landraces in this cluster. Cluster IV displayed above average value for majority of the quantitative traits except for internode length, leaf width, panicle length and length of primary branches. Landraces like*,* Keteto, Tikureta as well as some Degalet and Jamyo types were grouped in this cluster. South Welo, North Welo and North Shewa together accounted for more than 87, 84, 92 and 83% of the landraces in Cluster I, II, III and IV, respectively.

The landraces in clusters V, VI, VII and IX showed below average values for most of the quantitative traits. Clusters V and VII include most of Jigurte, Cherekit and other early maturing types where North Welo accounted for 55% of the landraces in Cluster V while South Welo accounted for 51% of the landraces in Cluster 6. White, light red and light brown seed colors were equally important in cluster V accounting for more than 65% of the landraces in the cluster. The sweet stalk sorghums were included in cluster VI. Cluster VII contained all improved varieties, landraces from North Welo and Oromiya. White seed color was dominant in this cluster. Cluster VIII contained mixture of landraces, where more than 54% were landraces from South Welo. The landraces in cluster IX include open panicle and small seeded landraces like Inchiro, Wancho*,* Wofaybelash, Merere and Slimo. This cluster had the smallest 1000 seed weight (27.7 g), and many of them (44%) are from South Welo. Light brown seed color predominates the group, and many of the landraces have seeds partly covered with glumes. Cluster X contained majority of landraces known as Zengada, a type of landrace widely adapted but mainly grown in higher altitude areas, and South Welo accounted for 54% the landraces in the group. These landraces were very late maturing with lower panicle weight, grain weight per panicle and 1000 seeds weight. Red brown seed color, semi-loose and semi-compact panicles, completely starchy endosperm and grey glume color manifested most of the landraces in this cluster.

The clustering of zones revealed the close relationship between landraces from North Welo and South Welo; from North Shewa and Oromiya (Figure 2B). The similarity values ranged from 0.24 between South Welo and Improved varieties to 0.93 between North Welo and South Welo. Landraces from Harerge showed close relation with landraces from North Shewa and Oromiya relative to other zones. The improved varieties formed a separate cluster. At district level, the grouping appeared to reflect both geographical proximity and ecological similarity (Figure 2C) with similarity values ranging from 0.72 between Artuma Jile and Improved varieties to 0.82 between Kobo and Habru. Landraces from Kobo, Habru, Artuma Jile and Bati grouped closely. Closer to this group was the grouping of landraces from Gubalafto, Shewa Robit, Ambassel, Tehuledere and Kalu. These districts belong to the lowland and intermediate altitude agro-ecologies. Landraces from high altitude districts such as Dessie zuria, Debressina and Merabete were relatively closely related. The improved varieties grouped distinctively from other districts.

Principal component analysis

Principal component analysis showed that the first five components with Eigen values greater than unity explained 71% of the variability among the landraces (Table 5). The first component was correlated mainly with days to flowering and maturity, followed by leaf number and leaf length.

The second component was associated with panicle weight, grain weight per panicle and grain number per panicle, and the third component with panicle length and length of primary branches. The fourth component correlated with 1000-seeds weight while the fifth component mainly correlated with internode length. Figure 3 shows the principal component loadings for the 17 quantitative traits and the ordination (grouping) of the landraces. Landraces opposite side of trait vector arrows indicate small value for the particular trait while those landraces on the direction of vector arrows display high values. The length of the vectors is proportional to the magnitude of the trait in grouping the landraces.

DISCUSSION

The descriptive, ANOVA, cluster and principal component analyses revealed the presence of high phenotypic variability among the sorghum landraces from the study area at large as well as within each of the four zones. The analyses for quantitative traits revealed wide variability among the landraces. Previous studies (Teshome et al., 1999; Seboka and van Hintum, 2006; Shewayrga et al., 2008) reported that farmers purposely maintain and grow many landraces to address various needs as well as risk aversion strategy, and the landraces vary in maturity, yield potential, stress tolerance, end-use quality and other agronomic traits. Diversity studies in NE Ethiopia have also shown high

Table 5. Principal component matrix showing Eigen values, variance and Eigen vectors for 17 quantitative traits in sorghum landraces.

Traits	Component				
	1	2	3	4	5
DF	**0.89**	0.05	0.06	-0.19	-0.13
DM	**0.90**	0.06	0.05	-0.12	-0.11
LN	**0.80**	0.25	-0.12	-0.05	0.02
LL	**0.71**	0.25	0.01	0.22	0.09
LW	**0.43**	0.41	0.13	-0.27	-0.13
IL	-0.10	0.04	-0.12	0.01	**0.90**
LSL	**0.47**	-0.05	0.35	0.18	0.43
PH	**0.66**	0.28	0.01	0.12	0.48
PE	-0.12	-0.19	0.22	0.44	0.25
PL	0.12	-0.05	**0.90**	0.06	-0.04
NPB	0.32	0.36	-0.21	0.25	-0.09
LPB	-0.10	0.00	**0.87**	-0.24	-0.03
PW	0.20	**0.92**	-0.03	0.21	0.01
GW	0.17	**0.93**	-0.08	0.27	0.04
SW	0.08	0.23	-0.13	**0.83**	-0.08
ThP	-0.18	0.32	-0.27	0.49	0.13
GN	0.16	**0.94**	-0.01	-0.13	0.08
Eigen values	3.88	3.27	1.93	1.60	1.38
percentage of total variance	22.84	19.25	11.34	9.42	8.14
Cumulative % of variance	22.84	42.09	53.43	62.85	70.99

DF=Days to 50% flowering, DM=Days to 90% maturity, LN=Leaf number, LL=Leaf length(cm) , LM=Leaf width (cm), IL= Internode length(cm) , LSL=Leaf sheath length(cm), PH=Plant height(cm), PE=Peduncle exertion(cm), PL=Panicle length(cm), NPB=Number of primary branches per panicle, LPB=Length of primary branches(cm), PW=Panicle weight(g), GW=Grain weight per panicle(g), SW=1000 seeds weight(g), ThP=Threshing percent (%), GN=Grain number per panicle.

diversity for other crop landraces including tef (Assefa et al., 2001; Kefyalew et al., 2000), barley (Abebe et al., 2010; Mekonnon et al., 2015) and durum wheat (Eticha et al., 2005; Mengistu et al., 2015). Generally, NE Ethiopia is one of the crop diversity areas of the country attributed to the wide topographic and agro-ecological variation coupled with subsistence farming requiring landraces that are locally adapted to marginal environments.

Although, there was high variability for most of the qualitative traits, some trait classes were more frequent than others. For example, most of the landraces were non-juicy, had starchy endosperm, compact panicles and 25% glume covered grain. Compact panicle, an adaptive trait, is a character of durra race mainly grown in dry areas (Doggett, 1988; Stemler et al., 1977). These types of landraces are the most preferred types by farmers for qualitative and quantitative attributes as well as end use. The predominance of starchy types may be attributed to farmers' intentional selection for suitability for *Injera,* the staple bread (Gebrekidan and Gebrehiwot, 1982). Besides, the landraces showed low diversity for grain covering and stalk juiciness at all levels of geographic domains. Majority of the landraces were with only 25% of the grain covered. Previous studies (Ayana and Bekele, 1998) observed increasing trend of 25% grain covered from high rainfall areas of Western Ethiopia to dry areas of Eastern Ethiopia. The same was true for compact panicle types. Grain cover by glumes is related to threshability and seed size, which are important sorghum selection criteria for farmers of the area (Teshome et al., 1999). It is also an adaptive trait where it plays important role in reducing grain mold in high rainfall and humid areas like Western Ethiopia. NE Ethiopia is dryland area with regular moisture stress (deficit) to crops where grain mold in the field is not a serious problem. Consequently, landraces with 50% or more grain covered with glume were not important among the landraces evaluated. This result is in line with previous observation in farmers' fields (Shewayrga et al., 2008). Such landraces were not widely grown and most of them were small seeded (1000-seeds weight) with brown color and often with very loose or open panicle. Some of these landraces such as Chobe and Ganseber are mainly for utilization of secondary importance like tella, genfo, while some others (e.g. Inchiro) are adapted to marginal environments. Farmers also reported some of these types as volunteers resulted from cross-pollination with wild types (Kilo). The juicy stalk sorghums are maintained for chewing purpose

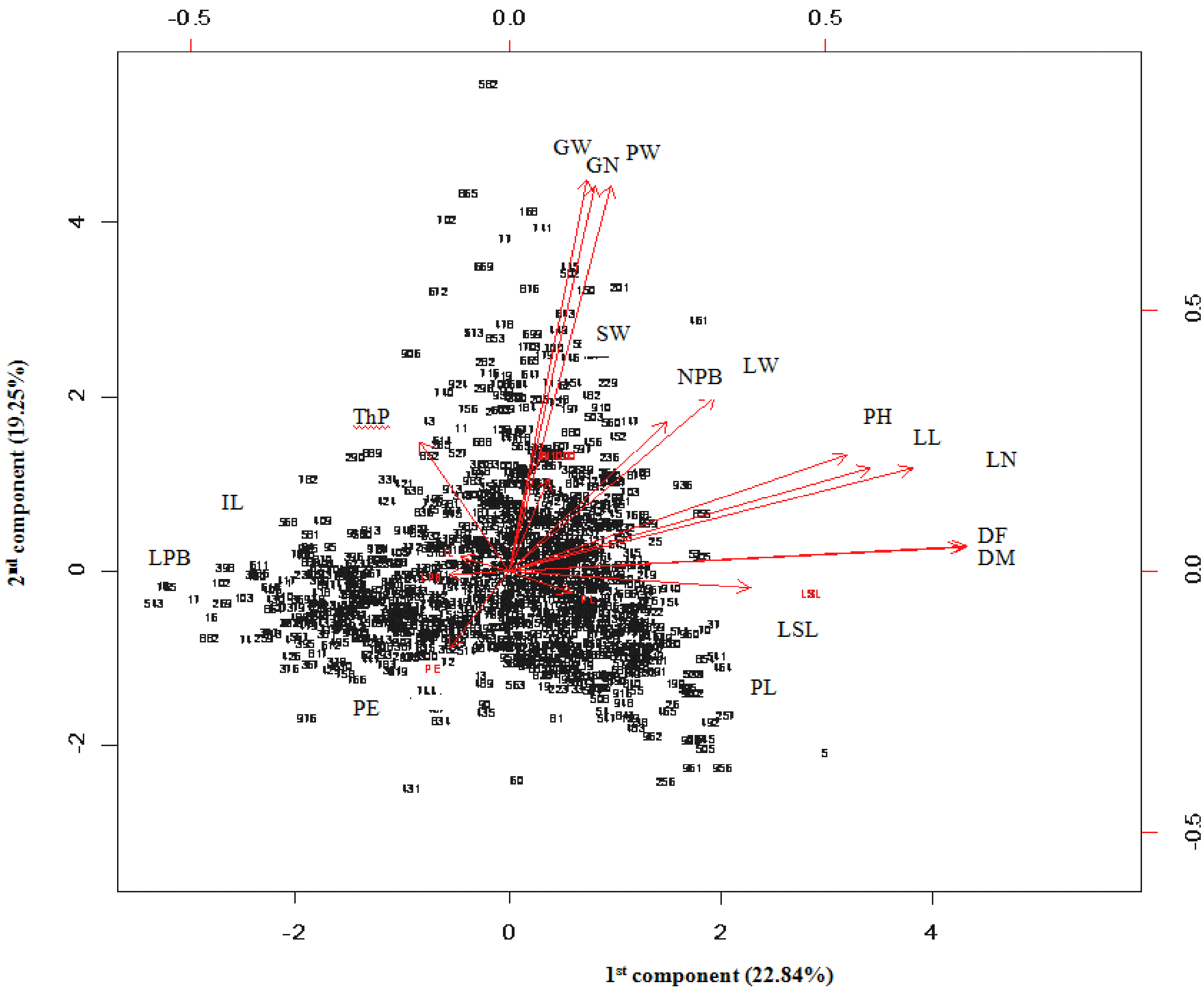

Figure 3. Biplot of landraces and traits for the first and second principal components. DF=Days to 50% flowering, DM=Days to 90% maturity, LN=Leaf number, LL=Leaf length(cm) , LM=Leaf width (cm), IL= Internode length(cm) , LSL=Leaf sheath length(cm), PH=Plant height(cm), PE=Peduncle exertion(cm), PL=Panicle length(cm), NPB=Number of primary branches per panicle, LPB=Length of primary branches(cm), PW=Panicle weight(g), GW=Grain weight per panicle(g), SW=1000 seeds weight(g), ThP=Threshing percent (%), GN=Grain number per panicle.

where farmers usually mix plant few sweet sorghum seeds in normal (non-juicy) sorghum fields. The farmers decision as to which landraces to plant, where and at what proportion involves a number of selection criteria to avoid risk, and the decision is focused mainly on normal types. This could explain the low variability observed for stalk juiciness.

Cluster analysis grouped the landraces into ten clusters, each having a wide within cluster variation. However, the clustering did not quite well correspond with geographic origin (administrative boundary) of the landraces. Rather, the grouping appeared to follow similarity in altitudinal and environmental factors as well as spatial proximity. The districts with close proximity and similar agro-ecology clustered together. Districts from lowland and intermediate altitudes have warmer climates as compared to those in higher altitudes. The physical distances and climatic factors may enforce physical and adaptive barriers to gene flow between agro-ecologically less similar and distant districts. The improved varieties formed separate cluster. These varieties were early maturity, short stature and most have genetic background from exotic origin. Ayana et al. (2000) also observed separate grouping of sorghum lines of exotic origin from Ethiopian landraces. The high morphological (phenotypic) variability among the landraces is an important resource to utilize the benefit of sorghum breeding of the area. The breeding focus to develop early maturing varieties for moisture stress areas does not appear to be fully effective as the improved varieties are very limited in number and area coverage in the study area (Shewayrga et al., 2008; Seboka and van Hintum, 2006). The improved varieties do not have the qualities the farmers are looking for in a variety (Seboka and van Hintum, 2006), which not only include yield but stalk yield, food making quality and other attributes. Integrating the attributes of important landraces in the breeding programs by identifying valuable parents with various traits of economic interest would be important for increasing the adoption rate of improved varieties.

In summary, the landraces displayed high variability for both quantitative and qualitative traits. But the differentiation among landraces from different geographical domains (zones, altitudes and districts) of the area was generally weak. Seed exchanges among farmers may attribute to frequent gene flow across the region resulting to weak differentiation. Concerning germplasm conservation, the weak differentiation among geographic domains and the high level of landraces variation observed at different levels of geographic domains suggest that a single large random collection from the whole target area would capture most of the genetic variation present in the sorghum landraces. However, targeted collection would also be important to capture specific but potentially valuable variability as landraces between districts showed appreciable differentiation. Besides, farm survey of landraces (Shewayrga et al., 2008) indicated a shift in sorghum types cultivated in the area where old and preferred landraces were either lost or marginalized in many localities while new types are coming into the system. Therefore, periodic collection surveys would be important to capture new variability.

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The first author acknowledges Sirinka Agricultural Research Center and Amhara Region Agricultural Research Institute, Ethiopia for the provision of research facilities, financial support and other necessary assistance throughout the field study, and the Ethiopian Institute of Biodiversity Conservation for provision of germplasm.

REFERENCES

Abdi A, Bekele E, Asfaw Z, Teshome A (2002). Patterns of Morphological Variation of Sorghum (*Sorghum bicolor* (L.) Moench) Landraces in Qualitative Characters in North Shewa and South Wello, Ethiopia. Hereditas 137(3):161-172.

Abebe TD, Bauer AM, Léon J (2010) Morphological diversity of Ethiopian barleys (*Hordeum vulgare* L.) in relation to geographic regions and altitudes. Hereditas 147:154-164.

Assefa A, Labuschagne MT (2004). Phenotypic variation in barley (*Hordeum vulgare* L.) landraces from north Shewa in Ethiopia. Biodiversity Conserv. 13:1441-1451.

Assefa K, Tefera H, Merker A, Kefyalew T, Hundera F (2001). Quantitative trait diversity in tef [*Eragrostis tef* (Zucc.) Trotter] germplasm from Central and Northern Ethiopia. Gen. Resour. Crop Evol. 48:53-61.

Ayana A, Bekele E (2000). Geographical patterns of morphological variation in sorghum (*Sorghum bicolor* (L.) Moench) germplasm from Ethiopia and Eritrea: quantitative characters. Euphytica 115:91-104.

Ayana A, Bekele E (1998). Geographical patterns of morphological variation in sorghum (*Sorghum bicolor* (L.) Moench) germplasm from Ethiopia and Eritrea: qualitative characters. Hereditas 129:195-205.

Brown AHD (1995). The core collection at the crossroads. In: Hodgkin T, Brown AHD, van Hintum ThJL, Morales EAV (eds.). Core collection of Plant Genetic Resources, IPGRI pp. 3-19.

Core Team R (2010). R: A language and environment for statistical computing. R foundation for statistical computing.

CSA (2003). Report on forecast of area and production of major crops in 2003/04. Federal Democratic Republic of Ethiopia, Central Statistical Authority (CSA). Bulletin P 283.

Doggett H (1988). The sorghum crop. Sorghum (2nd ed). Longman Group UK Limited, London, UK pp. 368-404.

Dossou-Aminon I, Loko LY, Adjatin A, Ewédjè EBK, Dansi A, Rakshit S, Cissé N, Patil JV, Agbangla C, Sanni A, Akoègninou A, Akpagana K (2015) Genetic Divergence in Northern Benin Sorghum (Sorghum bicolor L. Moench) Landraces as Revealed by Agromorphological Traits and Selection of Candidate Genotypes. The Sci. World J. http://dx.doi.org/10.1155/2015/916476

Eticha F, Bekele E, Belay G, Bo¨rner A (2005). Phenotypic diversity in tetraploid wheats collected from Bale and Wello regions of Ethiopia. Plant Gen. Resour. 3(1):35-43.

Gebeyehu G (1993). Characterization and evaluation of sorghum (*Sorghum bicolor* (L.) Moench) germplasms collected from Gambella. MSc Thesis. Alemaya University of Agriculture, Ethiopia.

Gebrekidan B (1973) The importance of the Ethiopian sorghum germplasm in the world collection. Econ. Bot. 27:442-445.

Gebrekidan B (1982) Utilization of germplasm in sorghum improvement. *In*: Sorghum in the Eighties: Proceedings of the International Symposium on Sorghum, 2-7 Nov. 1981. ICRISAT, India pp. 335-345.

Gebrekidan B, Gebrehiwot B (1982). Sorghum injera preparations and quality parameters. In: Proceedings of the International Symposium on Sorghum Grain Quality, 28-31 October 1981, Patancheru, AP, India. ICRISAT, Patancheru, AP India pp. 55-66.

Govindaraj M, Vetriventhan M, Srinivasan M (2015) Importance of Genetic Diversity Assessment in Crop Plants and Its Recent Advances: An Overview of Its Analytical Perspectives. Gen. Res. Int. http://dx.doi.org/10.1155/2015/431487

Grenier C, Bramel PJ, Dahlberg JA, El-Ahmadi A, Mahmoud M, Peterson GC, Rosenow DT, Ejeta G (2004). Sorghums of the Sudan: analysis of regional diversity and distribution. Gen. Resour. Crop Evol. 51:489-500.

Gerrano AS, Labuschagne MT, van Biljon A, Shargie NG (2014). Genetic diversity assessment in sorghum accessions using qualitative morphological and amplified fragment length polymorphism markers. Sci. Agric. 71(5):345-355.

Harlan JR, De Wet JMJ (1972) A simplified classification of cultivated sorghum. Crop Sci. 12(2): 172-176.

Haussmann BIG, Obilana AB, Ayiecho PO, Blum A, Schipprack W, Geiger HH (1999). Quantitative-genetic parameters of sorghum [*Sorghum bicolor* (L.) Moench] grown in semi-arid areas of Kenya. Euphytica 105:109-118.

Hayward MD, Sackville Hamilton NR (1997). Genetic Diversity: Population Structure and Conservation. In: Callow JA, Ford-Lloyd BV, Newbury HJ (Eds.). Biotechnology and plant genetic resources-Conservation and uses. CAB Int. pp. 49-76.

IBPGR/ICRISAT (1993). Descriptors for sorghum (*Sorghum bicolor* (L.) Moench). International Board for Plant genetic Resources, Rome, Italy; Int. Crops Res. Institute for the Semi-Arid Trop. Patancheru India.

Kahilainen A, Puurtinen M, Kotiaho JS (2014) Conservation implications of species–genetic diversity correlations. Global Ecol. Conserv. 2:315-323.

Kassahun B, Bidinger FR, Hash CT, Kuruvinashetti MS (2010) Stay-green expression in early generation sorghum [*Sorghum bicolor* (L.) Moench] QTL introgression lines. Euphytica 172 (3):351-362

Kebede Y (1991). The role of Ethiopian sorghum germplasm resources in the national breeding programmeme. *In*: Engels JMM, Hawkes JG and Worede M (eds.) Plant Gen. Resour. Ethiopia pp. 315-322.

Kefyalew T, Tefera H, Assefa K, Ayele M (2000). Phenotypic diversity for qualitative and phenologic characters in germplasm collections of tef (*Eragrostis tef*). Gen. Resour. Crop Evol. 47:73-80.

Kresovich S, Lamboy WF, McFerson JR, Forsline PL (1995). Integrating different types of information to develop core collections, with particular reference to *Brassica oleracea* and Malus x domestica. In: Hodgkin T, Brown AHD., van Hintum, ThJL and Morales EAV (eds.). Core collection of Plant Gen. Resour. IPGRI pp.147-154.

McGuire SJ (2002) Farmers’ Views and Management of Sorghum Diversity in Western Harerghe, Ethiopia: implications for collaboration with Formal Breeding. *In*: Farmers, Scientists and Plant Breeding (eds. Cleveland DA and Soleri D). CABI Publishing. pp. 107-135.

Mekonnon B, Lakew B, Dessalegn T (2015) Morphological diversity and association of traits in Ethiopian food barley (*Hordeum vulgare* L.) landraces in relation to regions of origin and altitudes. J. Plant Breed. Crop Sci. 7(2):44-54.

Mengistu DK, Kirosa AY, Pèb ME (2015). Phenotypic diversity in Ethiopian durum wheat (*Triticum turgidum* var. durum) landraces. The Crop J. 3:190-199.

Prasada Rao KE, Mengesha MH (1987). Morphology and distribution of Zerazera sorghums. J. Agric. Tradit. Bot. Appl. 34:51-55.

Ramanatha Rao VR, Hodgkin T (2002). Genetic diversity and conservation and utilization of plant genetic Resources. *Plant Cell,* Tissue and Organ Cult. 68:1-19.

Reddy VP, Upadhyaya HD, Gowda CLL (2006) Current status of sorghum genetic resources at ICRISAT: their sharing and impacts. J. SAT Agric. Res. 2:1.

Seboka B, Van Hintum T (2006). The dynamics of on-farm management of sorghum in Ethiopia: Implication for the conservation and improvement of plant genetic resources. Gen. Resour. Crop Evol. 53(7):1385-1403.

Shewayrga H, Jordan DR, Godwin ID (2008). Genetic erosion and changes in distribution of sorghum (*Sorghum bicolor* L. Moench) landraces in North-Eastern Ethiopia. Plant Genetic Resources: Charact. Util. 6(1):1-10.

Singh R, Axtell JD (1973) High lysine mutant gene (hl) that improves protein quality and biological value of grain-sorghum. Crop Sci. 13(5):535-539.

Stemler ABL, Harlan JR, de Wet JMJ (1977). The sorghums of Ethiopia. Econ. Bot. 31:446-460.

Subudhi PK, Rosenow DT, Nguyen HT (2000). Quantitative trait loci for the stay green trait in sorghum (*Sorghum bicolor* L. *Moench*): consistency across genetic backgrounds and environments. Theor. Appl. Genet. 101:733-741.

Tegegne G, Bandyopadhyay R, Mulatu T, Kebede Y (1994). Screening for ergot resistance in sorghum. Plant Dis. 78(9):873-876.

Teshome A, Fahrig L, Torrance JK, Lambert JD, Arnason TJ, Baum BR (1999). Maintenance of Sorghum (*Sorghum bicolor*, Poaceae) Landrace Diversity by Farmers' Selection in Ethiopia. Econ. Bot. 53(1):79-88.

Teshome A, Baum BR, Fahrig L, Torrance JK, Arnason TJ, Lambert JD (1997). Sorghum (*Sorghum bicolor* (L.) Moench) landrace variation and classification in North Shewa and South Welo, Ethiopia. Euphytica 97:255-263.

Vavilov NI (1951). The origin, variation, immunity and breeding of cultivated plants. Chron. Bot. 13:1-366.

Worede M (1992). Ethiopia: a genebank working with farmers. *In:* Cooper D, Vellve R, Hobbelink H (Eds.) Growing Diversity pp. 78-94.

Wu YQ, Huang Y, Tauer CG, Porter DR (2006) Genetic diversity of sorghum accessions resistant to greenbugs as assessed with AFLP markers. Genome 49:143-149.

academicJournals

Vol. 11(33), pp. 3123 -3133, 18 August, 2016
DOI: 10.5897/AJAR2016.11329
Article Number: D9F181560044
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Estimate of genetic parameters in bioactive and micronutrients compounds of maize

Ivan Ricardo Carvalho[1]*, Maicon Nardino[1], Alan Junior de Pelegrin[1], Jéssica Fernanda Hoffmann[1], Sonia Mendonça Poleto[1], Mauricio Ferrari[1], Vinicius Jardel Szareski[1], Daniela Meira[2], Fabio Clasen Chaves[1], Velci Queiróz de Souza[3], Antonio Costa de Oliveira[1] AND Luciano Carlos da Maia[1]

[1]Federal University of Pelotas, Pelotas, Rio Grande do Sul, 96010-165, Brazil.
[2]Federal University of Santa Maria, Frederico Westphalen, Rio Grande do Sul, 98400-000, Brazil.
[3]Federal University of Pampa, Dom Pedrito, Rio Grande do Sul, 96450-000, Brazil.

Received 17 June, 2016; Accepted 14 July, 2016

Maize *(Zea mays* L.) has a range of uses, from animal to human feed and can be consumed *in nature*, or as feedstock material in the preparation of starches, flours, canjicas, breads, beverages and porridges. This study aimed to determine the linear associations, estimates of genetic parameters and heterosis of bioactive and micronutrients compounds in maize. The experimental design was randomized blocks, the treatments consisted of six open pollinated varieties (OPVs) of maize: Dente de Ouro Roxo (P_1), BRS Missões (P_2), Caiano Rajado (P_3), AL 25 (P_4), Bico de Ouro (P_5), Argentino Branco (P_6) and five hybrids maize derived from crosses, P_2 x P_1, P_3 x P_1, P_4 x P_1, P_5 x P_1 e P_6 x P_1. Linear high and positive associations are expressed between the seed width and hundred kernel weight, between the antioxidants DPPH and ABTS radical, and through the manganese and zinc. The genetic parameters determine the progenies and the parent-average relationship are superior to the length, width and acidity of seeds, total phenols and copper. The heritability in a wide sense expressed is greater for the traits: acidity seeds, antioxidant DPPH radical and copper. The heritability in a restricted sense is superior to the traits length, width and acidity of seeds, carotenoids and sodium content. The traits thickness and color of seeds, total phenols, antioxidant ABTS radical and zinc are increased through the effects of intervarietal heterosis in maize. Estimates of genetic parameters obtained can be used in genetic maize breeding programs, in order to obtain biofortified genotypes using bioactive compounds and micronutrients.

Key words: *Zea mays* L., enriched cereals nutritionally, genetic variability, plant breeding.

INTRODUCTION

Maize (*Zea mays* L.) is in the family of Poaceae, with agricultural, social and economic importance. In Brazil, its cultivation covers the various producing regions, where emphasis is laid on increasing productivity per unit area. This cereal has a range of uses, which range from animal and human feed, and can be consumed in nature, or as feedstock material in the preparation of starches, flours, canjicas, breads, beverages and porridges (Estrada et al., 2014). Thus, in many parts of Africa, Mexico and Brazil, maize is a source of energy and mineral, affordable and low cost to the population (Paes, 2006). Currently, the programs of genetic improvement of maize recommend obtaining superior genotypes, efficiently improving management techniques available (Ribeiro et al., 2016).

In this context, to minimize the effects of the food and nutritional deficiency, and prevent cardiovascular and chronic diseases non transmissible as cancer in low-income populations (Costa et al., 2013), an efficient alternative and economically variable is direct breeding programs to obtain maize genotypes enriched by bioactive and micronutrients compounds (Zilic et al., 2016). Bioactive compounds (carotenoids and flavonoids) are derived from secondary plant metabolism (Silva et al., 2010), confer to the food, the antioxidant capacity that minimizes the negative effects of reactive oxygen species and nitrogen (Wen et al., 2012; Costa et al., 2013), reducing the harmful free radicals to the human organism (Sikora et al., 2008). In order to obtain superior genotypes for bioactive and micronutrients compounds, it is necessary to search for greater genetic variability for traits of interest (Cardoso et al., 2009), thus, the use of open pollinated varieties (OPVs) presented variable to clarify the needs of improving the culture (Setimela et al., 2007). However, it is possible that the obtaining of intervarietal hybrid can contribute substantially to the increase of these compounds. With the lack of information regarding the genetic parameters of these traits, this study aimed to determine the linear associations, estimates of genetic parameters and heterosis of bioactive and micronutrients compounds in maize.

MATERIALS AND METHODS

The experiment was conducted in 2015 at Genomics Center and Plant Breeding of the Federal University of Pelotas. The seeds were obtained in the 2014/2015 crop year in the Agricultural Center of Palma in Capão do Leão-Brazil, in latitude 31 47 '58' 'S and longitude 52° 31' 02 '' O, with an altitude of 13.2 m. The soil is characterized as Argisol red yellow dystrophic. The experimental design was randomized blocks with 11 treatments arranged in three replications. The treatments consisted of six open pollinated varieties (OPVs) of maize: Dente de Ouro Roxo (P_1), BRS Missões (P_2), Caiano Rajado (P_3), AL 25 (P_4), Bico de Ouro (P_5), Argentino Branco (P_6), and five hybrids maize derived from crosses, P_2 x P_1, P_3 x P_1, P_4 x P_1, P_5 x P_1 e P_6 x P_1.

The seeds were stored in cold chamber at ±4°C for 180 days, with homogeneity 13% humidity. Subsequently, the samples were subjected to the cleaning to the exclusion of strange particles, 500 g of seed per genotype was obtained, which were homogenized again and the measurements of the pre-grinding of the seeds traits was done. After the seeds were crushed in Marconi® MA 020 mill fitted with a 0.053 mm sieve, the ground sample of each genotype was divided into three sub-samples of 150 g, being directed to the Secondary Metabolism Laboratory UFPel, Brazil.

The measured traits in pre-grinding were: seed length (SL), seed width (SW), seed thickness (ST) both expressed in mm and measured with the assistance of a digital paquimeter in 90 seeds per genotype; hundred kernel weight (HW), in grams. The measured traits in post-grinding were: seed color (SC) in Hue angle; seed acid (AC) in percent of citric acid; pHin hydrogenionic potential; soluble solids (SS) in °Brix (AOAC, 2005), total phenols (TP) in µg g^{-1} (Singleton and Rossi,1965); total flavonoids (TF) in mg g^{-1} (Zhishean et al., 1999); total carotenoids (TC) in mg g^{-1} (AOAC, 2005); antioxidant potential by DPPH (DP), as a inhibition percentage (Brand-Williams et al., 1995); antioxidant potential by ABTS radical (AB), in inhibition percentage (Rufino et al., 2007). The micronutrients: copper (Cu), zinc (Zn), sodium (Na), manganese (Mn) and iron (Fe) were expressed in mg kg^{-1} (Tedesco et al., 1995).

The data were submitted to analysis of variance at 5% probability in order to verify their presuppositions, the normality test of Wilk (1965), and homogeneity of variances by Bartlett (Steel et al., 1996) was used. Subsequently, a descriptive analysis by the distributions of phenotypic frequencies, and in conjunction the parent average (PA) for the distinction of upper and lower classes were used. Pearson correlation was performed in order to show the trend of associations among traits where their coefficients were based on the classification of Carvalho et al. (2004). The data were standardized by the rating of Z scores, as:

$$Z=\frac{\mathbf{xi}-\boldsymbol{\mu}}{\theta}$$

Where *xi:* is the observed value, *µ*: corresponds to the average, and *θ:* the standard deviation of the trait (Cruz et al., 2014). The genetic parameters were obtained by the father-average regression method and progenies (covariance of $\frac{1}{2}$ additive variance), and the angle β coefficient corresponds to the total genetic variance (Falconer, 1987). The father-average correlation with the progenies was estimated (*r*), the phenotypic variance (*Vp*), additive variance (*Va*), heritability in the broad sense (H^2), heritability in the narrow sense (h^2) and heterosis percentage were estimated with the methodology proposed by Ramalho et al. (2012). The analyses were performed using Excel and Genes software (Cruz, 2013).

RESULTS AND DISCUSSION

Analysis of variance for traits

Analysis of variance revealed significant difference at

*Corresponding author. E-mail: carvalho@gmail.com.

P>0.05 probability for the 18 traits measured in open pollinated varieties (OPVs) and maize hybrids. The coefficients of variation (CV%) obtained in the experiment presented range of 0.74 to 19.76% and showed accuracy in conducting the experiment, transmitting reliability of the data. Thus, nine traits obtained CV less than 10% with high precision, others are evident in the range of 10 to 20% being ranked with good experimental precision (Gomes, 2000).

Descriptive analysis by the distributions of phenotypic frequencies

The hybrids maize were submitted to descriptive analysis by frequency distributions, in order to reveal the number and amplitude of the phenotypic classes formed, besides showing that these comprise the largest number of genotypes. The seed length (SL) formed five classes with amplitude of 9.9 to 12.9 mm (Figure 1). Classes of 10.5 and 12.3 mm comprise 66.7% of hybrids maize, however, the overall parents average (PA: 12.10 mm) discriminated classes 12.3 and 12.9 mm with 46.7% upper progenies due to intervarietal crossing. The seed width (SW) formed four classes with amplitude of 9.2 to 10.8 mm (Figure 1B). The classes of 9.2 and 9.6 mm comprise 73.3% of hybrids maize, and the overall average of the parents (PA: 9.78 mm) points out that the class of 10.0 and 10.8 mm have 26.6% of the upper progenies.

The seed thickness (ST) formed five classes with amplitude of 4.0 to 5.6 mm (Figure 1C). The class of 4.8 mm corresponds to 40% of hybrids maize, and the overall parents average (PA: 4.37mm), revealed that the classes of 4.4; 4.8; 5.2 and 5.6 mm included 93.3% of the superior progenies. Research has shown that the dimensions of maize seeds only changed the initial establishment of plants, morphological and productivity are not influenced (Vazquez et al., 2012). In another report, effects of seed size on maize physiological characteristics and seedling growth were not observed (Sangoi et al., 2004). The hundred kernel weight (HW) formed five classes with amplitude of 28 to 44 g (Figure 1D). The class with 32 g included 33.3% of hybrids maize, and the overall parents average (PA: 37.9 g) distinguished the classes of 40 and 44 g with 26.7% of the superior progenies. Crossings intervarietal in partial diallel with 18 OPVs obtained 96 hybrids of maize where 46.1% increased hundred kernel weight from the effects of heterosis (Oliveira et al., 2007). Research determined that regardless of the type of intersection that get simple hybrid of maize, double or triple, direct and positive associations are consolidated among the hundred kernel weight, kernel weight of ear and productivity (Lopes et al., 2007; Nardino et al., 2016).

The seed color (SC) formed six classes with amplitude of 89.2 to 96.7 of the Hue angle (Figure 1E). The class of 95.2 corresponded to 33.3% of hybrids maize which can be classified as yellow (Hue angle ± 90), the overall parent average (PA: 84.37 Hue) is intermediate to red colorations (Hue angle<90) and yellow (Hue angle ± 90), where all intervarietal crossings provided increase in the color of the seeds with superior progenies angle Hue to parents. The color of maize seeds is due to the proportion of existing carotenoid (Kimura et al., 2007). The most frequent carotenoids in maize are lutein, zeaxanthin and β-carotene (Silva et al., 2010). The seed acid (AC) formed five phenotypic classes with amplitude of 0.21 to 0.45% (Figure 1F). The class with 0.33% showed 53.3% of hybrids maize, and the overall parents average (PA: 0.30%) distinguished classes of 0.33; 0.39 and 0.45% acidity with 66.7% of the progeny superior to parents. The seeds pH (pH) formed with four classes amplitude from 6.3 to 6.9 (Figure 1G). The class of 6.3 is higher with 73.3% of hybrids maize, and the overall parents average (PA: 6.64) revealed that the classes of 6.8 and 6.9 obtained 20% of the superior progenies. The acidity is inversely proportional to pH, with the higher acidity of the fruit, conducive for higher respiratory rate of plant stages, in physiological maturity, the acidity tends to decrease due to the decrease in metabolic activity (Junior et al., 2008). In maize, the major metabolic rates are obtained during the reproductive period after fertilization of the ovules, which coincides with the definition of the magnitude of seeds per ear (Argenta et al., 2003).

The soluble solids (SS) formed five classes with amplitude of 2.4 to 7.2 °Brix (Figure 1H). The class of 3.6 °Brix is higher with 46.7% of hybrids maize, and the overall parents average (*PA*: 4.66 °Brix) distinguish classes of 4.8, 6.0 and 7.2 °Brix with 40% of the superior progenies. The soluble solids in maize are directly influenced by the characteristics of genotype, photosynthetic efficiency of the plant and the water supply, where the largest proportions are expressed as milky seed in R_3 stadium. In this way, the increase in soluble solids provides larger dimensions and kernel weight (Magalhães and Durães, 2006). The total phenols (TP) formed five classes with amplitude of 660 to 960 µg g^{-1} (Figure 1I). The 720 µg g^{-1} class corresponds to 33.3% of hybrids maize, and the overall parents average (PA: 657.32 µg g^{-1}) indicated that all obtained progenies were superior to their parents due to heterosis of intervarietal crossings. Phenols are compounds of secondary metabolism and contribute positively to the antioxidant potential (Silva et al., 2010).

The total flavonoids (TF) formed five classes with amplitude of 400 to 800 µg g^{-1} (Figure 2A). The class of 400 µg g^{-1} is higher with 33.3% of hybrids maize, and the overall parents average (PA: 495.45 µg g^{-1}) discriminated classes 500, 600, 700 and 800 µg g^{-1} with 66.7% of the

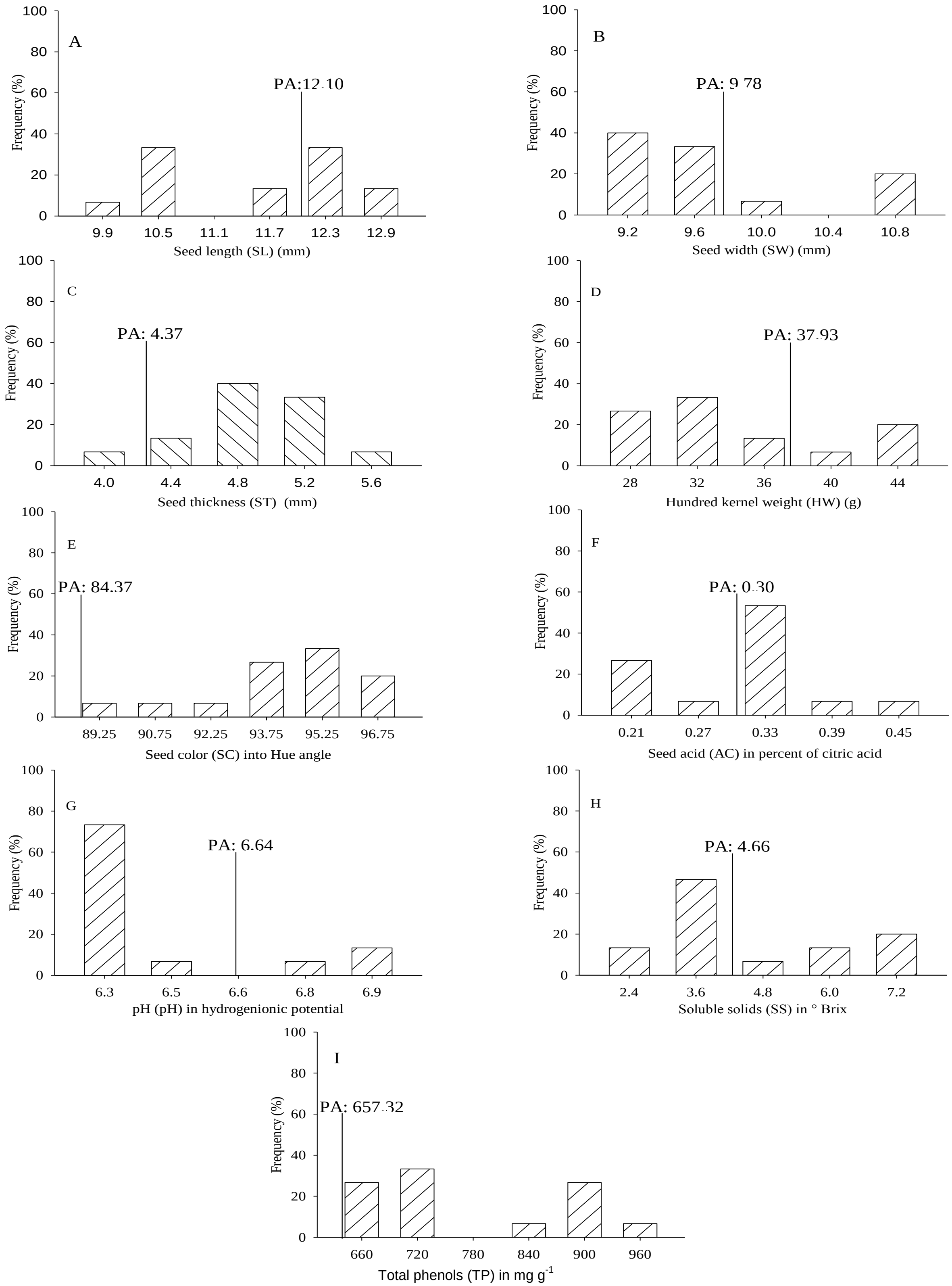

Figure 1. Frequency distributions of phenotypic classes for the traits: graphs (A) seed length (SL); (B) seed width (SW); (C) the seed thickness (s); (D) hundred kernel mass (HW); (E) seed color (CR); (F) seed acid (AC); (G) pH in hydrogenionic potential (pH); (H) soluble solids (SS); (I) total phenols (TP), measured in hybrids maize.

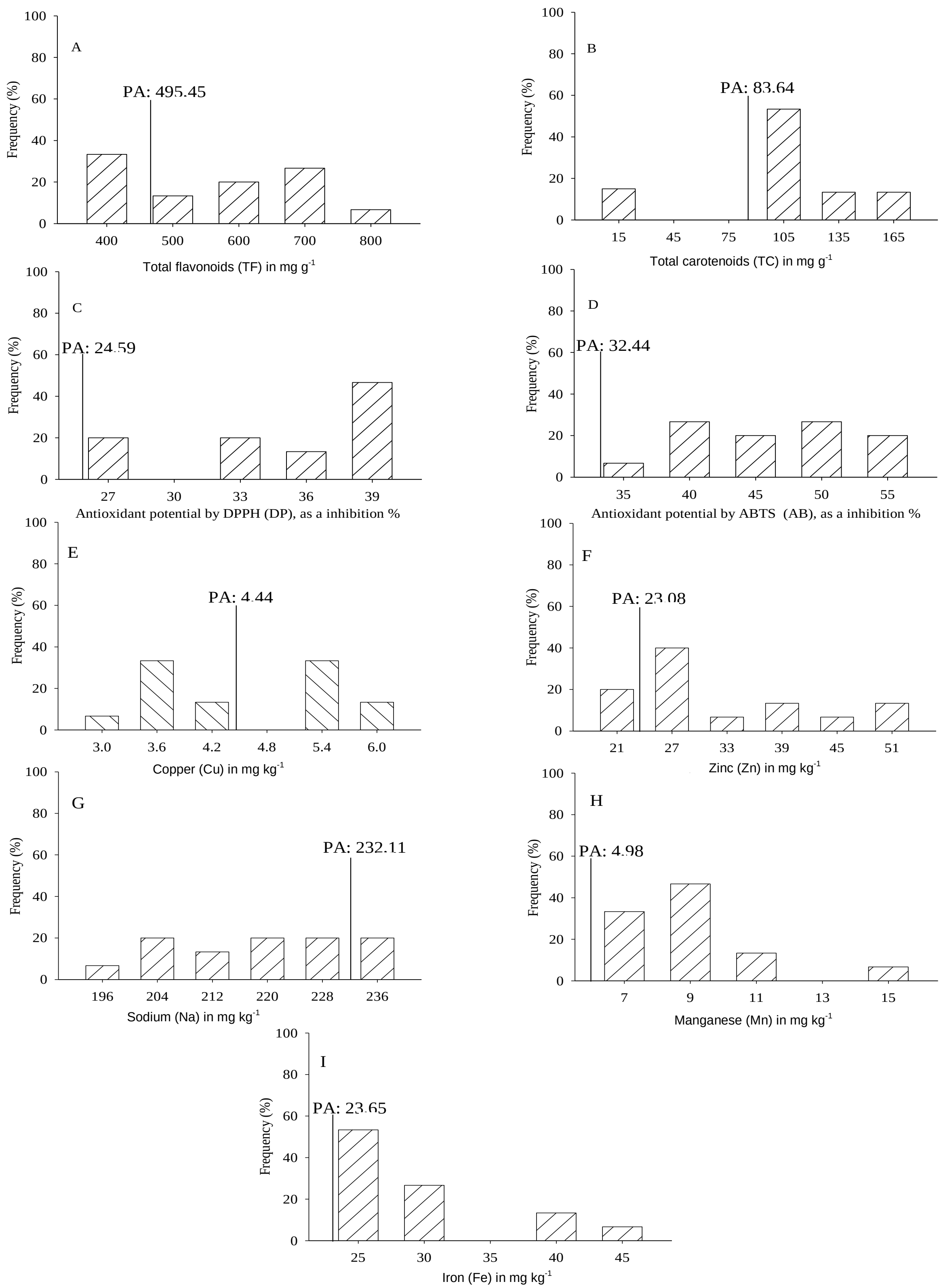

Figure 2. Frequency distributions of phenotypic classes for the traits: graphs (A) total flavonoids (TF); (B) total carotenoids (TC); (C) antioxidant potential by DPPH radical (DP); (D) antioxidant potential by ABTS radical (AB); (E) copper (Cu); (F) zinc (Zn); (G), sodium (Na); (H) manganese (Mn); (I) iron (Fe), measured in hybrids maize.

superior progenies. The flavonoids contributed to the development of the plant and assist in protecting the tissues to light stress, the incidence of insect pests minimize the free radicals by acting as antioxidants, regulating the hormonal and enzymatic balance, thus, searching maize define the exogenous application of flavonoid that influenced increased productivity (Oliveira and Fernandes, 2008). The total carotenoids (TC) formed four classes with amplitude of 15 to 165 µg g^{-1} (Figure 2B). The class with 105 µg g^{-1} is higher with 53.3% of hybrids maize, and the overall parents average (PA: 83,64µg g^{-1}) provided distinguished the classes of 105, 135 and 165 µg g^{-1} with 80% of the superior progenies. Studies carried out on 10 OPVs of maize revealed that total carotenoids are formed by larger fractions of xanthophylls, zeaxanthin, β-cryptoxanthin and β-carotene (Rios et al., 2012). The maize breeding is aimed increasing carotenoids in the seeds to obtain genotypes with higher content of provitamin A (Cardoso et al., 2009).

The antioxidant potential was obtained against DPPH (DP) formed in four classes of amplitude 27-39% inhibition of the radical (Figure 2C). The class with 39% inhibition corresponds to 46.7% of hybrids maize, and the overall parents average (PA: 24.59%) shows that all progenies are superior to parents. The antioxidant potential obtained against ABTS (AB) was formed with five classes with amplitude of 35-55% inhibition of the radical (Figure 2D). Classes of 40 and 50% were higher with 53.3% of hybrids maize, and the overall parents average (PA: 32.44%) shows that all progenies are superior to parents. The antioxidant activity minimizes free radicals and oxidative stress in humans, leading to compounds with antioxidant activity such as the carotenoids, phenols and flavonoids (Silva et al., 2010).

The content of copper (Cu) formed five classes with an amplitude of 3 to 6 mg kg^{-1}(Figure 2E). The classes of 3.6 and 5.4 mg kg^{-1} was higher with 66.7% of hybrids maize, and the overall parents average (*PA*: 4.44 mg kg^{-1}) discriminated classes of 5.4 and 6.0 mg kg^{-1} with 46.7% of the superior progenies. Cu is a constituent of enzymes and proteins, which operates in photosynthetic processes, respiration, electron transport, is a component of secondary metabolites primarily of phenols, and closely related to the development of seeds (Kirkby and Romheld, 2007). The content of zinc (Zn) formed six classes with amplitude of 21 to 51 mg kg^{-1} (Figure 2F). The 27 mg kg^{-1} class was higher with 40% of hybrids maize, and the overall parents average (PA: 23.08 mg kg^{-1}) distinguishes the class of 27, 33, 39, 45 and 51 mg kg^{-1} with 80% superior progenies. The Zn actively participates in the interactions between enzymes and substrates, helps tryptophan synthesis and auxin which are responsible for the growth of plant tissues, influence positively the carbohydrates, proteins and membranes (Kirkby and Romheld, 2007), elongation of the stem internodes, accumulation of biomass and seed yield in maize (Andreotti et al., 2001).

The sodium content (Na) formed six classes with amplitude of 196 to 236 mg kg^{-1} (Figure 2G). The hybrids maize have uniform distribution among the phenotypic classes, and the overall parents average (PA: 232.11mg kg^{-1}) distinguishes 236 mg kg^{-1} class with 20% of the superior progenies. The manganese (Mn) shows four classes formed with amplitude of 7 to 15 mg kg^{-1} (Figure 2H). The 9 mg kg^{-1} class stands higher with 46.7% of hybrids maize, and the overall parents average (PA: 4.98mg kg^{-1}) shows that all progenies were superior to parents. The Mn acts directly on photosynthetic processes with the breakdown of water and electron transport molecule, minimizes the effects of reactive oxygen species and is an enzyme cofactor that assists in secondary metabolism of phenols and flavonoids. In maize, it is crucial for male inflorescence, viability of anthers and pollen, and closely linked to the physiological potential of seeds (Kirkby and Romheld, 2007). The iron (Fe) formed four classes with amplitude of 25 to 45 mg kg^{-1} (Figure 2I). The class of 25 mg kg^{-1} is higher with 53.3% of hybrids maize, and the overall parents average (PA: 23.65 mg kg^{-1}) shows that all progenies were superior to their parents. The Fe comprises the proteins responsible for electron transport, is an enzyme activator that regulates the biosynthesis of chlorophyll and function of chloroplasts, in contrast, groups phenols fractions for lignification of tissues, especially the root (Kirkby and Romheld, 2007). Few genes control the iron accumulation in the seeds of efficiency, but are dependent on the phloem transport and the amount of minerals contained in the plant tissues (Rios et al., 2009).

Pearson linear correlation between traits

The Pearson correlation was performed in order to identify the tendency of associations among 18 traits measured in open pollinated varieties (OPVs) and intervarietal hybrids maize (*N*=33). 153 linear associations among traits, 42 significant at the P>0.05 probability was carried out (Table 1). The linear correlation coefficients followed the classification proposed by Carvalho et al. (2004). The seed length (SL) has intermediate and positive trend with HW (r=0.42), however, intermediate and negative trends with ST (r=-0.52), pH (r=-0.53) and SS (r=-0.41). Maize seeds with larger longitudinal dimensions tend to increase its weight and reduce the thickness by changing the compliance of the endosperm. In this way, the modifications affect the accumulation of total sugars and cause a decrease in pH. Smaller ears direct assimilated available to a smaller number of seeds, the increased kernel weight is due to the transformation

Table 1. Estimates of Pearson linear correlations for the traits linked to the seeds dimensions, bioactive compounds and micronutrients in open pollinated varieties (*OPV*s) and measured in hybrids maize.

Traits	**SL[(1)]**	**SW**	**ST**	**HW**	**SC**	**AC**	**pH**	**SS**	**TP**
SL[(1)]	-	0.30	-0.52*	0.42*	-0.27	0.12	-0.53*	-0.41*	-0.04
SW		-	0.25	0.84*	0.06	-0.25	-0.24	-0.50*	0.38*
ST			-	0.05	0.37*	-0.07	0.05	-0.06	0.50*
HW				-	-0.27	-0.12	-0.33	-0.45*	0.26
SC					-	-0.22	0.16	-0.14	0.27
AC						-	-0.13	0.02	0.07
pH							-	0.50*	-0.33
SS								-	-0.29
TP									-
Traits	TF[(1)]	TC	DP	AB	Cu	Zn	Na	Mn	Fe
SL[(1)]	-0.18	-0.12	-0.22	-0.14	-0.21	-0.30	0.14	-0.28	-0.20
SW	0.06	-0.07	0.12	-0.15	-0.04	-0.37*	-0.39*	-0.20	-0.20
ST	0.02	0.17	0.54*	0.30	0.24	0.19	-0.17	0.34	0.29*
HW	-0.20	-0.11	-0.05	-0.34	-0.15	-0.68*	-0.25	-0.48*	-0.39*
SC	0.30	0.31	0.52*	0.23	-0.04	0.44*	-0.27	0.54*	0.41*
AC	-0.05	0.18	0.31	0.38*	-0.42*	-0.35*	0.35*	-0.26	0.15
pH	-0.20	0.25	0.01	-0.04	0.01	0.14	0.05	-0.13	-0.04
SS	-0.28	0.53*	-0.25	-0.08	-0.03	0.03	0.34*	-0.10	0.06
TP	0.09	0.21	0.60*	0.37*	-0.17	-0.07	-0.17	0.26	0.07
TF	-	-0.43*	0.10	0.12	0.13	0.40*	-0.26	0.45*	0.30
TC		-	0.26	0.18	-0.53*	-0.18	0.10	-0.04	0.23
DP			-	0.69*	-0.23	0.16	-0.25	0.45*	0.32
AB				-	-0.16	0.38*	0.08	0.47*	0.19
Cu					-	0.43*	-0.13	0.23	0.11
Zn						-	-0.15	0.85*	0.40*
Na							-	-0.33	-0.20
Mn								-	0.42*
Fe									-

*Coefficients of Pearson linear correlations (n=33) significant at P>0.05 probability. [(1)]Seed length (SL); seed width (SW); seed thickness (ST); hundred kernel mass (HW); seed color (SC); seed acid (CA); pH in hydrogenionic potential (pH); soluble solids (SS); total phenols (TP); total flavonoids (TF); total carotenoids (TC); antioxidant potential by DPPH radical (DP); antioxidant potential by ABTS radical (AB); copper (Cu); zinc (Zn); sodium (Na); manganese (Mn); iron (Fe).

of soluble sugars in starch between the stages R_2 and R_6, which results in maximum dry matter accumulation in maize seed (Magalhães and Durães, 2006).

The seed width (SW) shows high positive trend with HW (r=0.84) and with the intermediate and positive TP (r=0.38), however, intermediate and negative with the SS (r=- 0.50), Zn (r=-0.37) and Na (r=-0.39). The results indicate that larger seeds have the greatest weight, and increase total phenolic concentration with decrease in the mineral fraction through the elements Zn, and Na. The seed thickness (ST) has intermediate and positive trends with SC (r=0.37), TP (r=0.50), DP (r=0.54) and Fe (r=0.29). Maize seeds when thick tend to express stronger coloration, increase the pigments contained in their integument, which is indicative of greater accumulation of phenolic compounds and carotenoids. In this way, genotypes with high phenolic fraction are superior to the antioxidant potential and iron accumulation in the seed. The antioxidant potential is closely related to the phenolic fraction of vegetable, where the largest proportion of carotenoids in maize (lutein and zeaxanthin) minimizes the negative effects of reactive oxygen species (Silva et al., 2010). Research indicates that OPVs of maize have higher concentrations of iron in the seeds as compared to hybrids and larger proportions of this mineral are contained in the embryo seeds with hard seed coat (Castro et al., 2009).

The hundred kernel weight (HW) reveals high and negative trend with Zn (r=-0.68), intermediate and negative with SS (r=-0.45), Mn (r=-0.48) and Fe (r=-0.39). Inverse associations between kernel weight and mineral buildup is justifiable because the endosperm is formed by larger proportions of starches carbohydrates, and the embryo assign the largest mineral fractions (Castro et al., 2009). Thus, the kernel weight increment does not show abrupt effect on the accumulation of minerals. In contrast, the increase in Zn and Fe ratios in maize seed can be achieved with efficient use of the best genitors, in order to explore the hybrid combination by additive and non-additive effects together with combine selection strategies that prioritize get genotypes with higher proportions of micronutrients and productivity (Menkir, 2008).

The color of the seeds (SC) expressed intermediate and positive trends with DP (r=0.52), Zn (r=0.44), Mn (r=0.54) and Fe (r=0.41). The seeds with more pigmented integument tend to have higher proportions of bioactive compounds with increased antioxidant activity and the ability to suppress reactive oxygen species (Silva et al., 2010). These effects are enhanced by Fe functionality to transport electrons, where Mn assists the secondary metabolism, Zn mainly in the synthesis of hormones auxin, all micronutrients of which are enzyme cofactors (Kirkby and Romheld, 2007). The seed acid (AC) showed positive tendencies and intermediate AB (r=0.38) and Na (r=0.35), however, intermediate and negative Cu (r=-0.42) and Zn (r=-0.35). The pH is associated with intermediate and positive SS (r=0.50). The pH is proportional to the fraction of soluble sugars and inversely to the acidity. Thus, the sodium accumulation in seeds coats resulted in increased acidity, and inhibit the ABTS radical, however, micronutrients Cu and Zn decrease. Research in coffee clarify the dynamics of associations of bioactive compounds where lower proportions of soluble sugars and proteins indicate increasing acidity, the phenolic compounds and therefore the antioxidant activity (Abrahão et al., 2010).

The soluble solids (SS) show intermediate and positive association with the TC (r=0.53), total phenols (TP) have intermediate positive trend with DP (r=0.60) and AB (r=0.37). The antioxidant potential obtained by DPPH radical expressed high positive association with ABTS radical (r=0.69). These combinations indicate linear dependence between the radicals and preliminarily, which can be explained through similar dynamics. For OPVs and hybrid of maize, antioxidant activity are attributed to total phenols, where most soluble solids fraction increases the total carotenoids (lutein, zeaxanthin, β-cryptoxanthin and β-carotene) (Kimura et al., 2007). Therefore, maize seed are more pigmented due to carotenoids which may have antioxidant activity.

Zn is associated with intermediate and positively with TF (r=0.40), AB (r=0.38) and Cu (r=0.43). While Mn presents highly positive trend with Zn (r=0.85), and intermediate and positive with TF (r=0.45), DP (r=0.45) and AB (r=0.47). In contrast, the Fe relates to intermediate and positive way with Zn (r=0.40) and Mn (r=0.42). By identifying traits which define the antioxidant potential in maize, it appears that both Fe and total flavonoids tend to increase Zn and Mn, and both inhibit ABTS radical, and contribute to antioxidant activity.

Parent-progeny regression and obtaining the estimation of genetic parameters

The relation of the progenies with the father-average (*r*) represents how the characteristic may be associated with offspring by crossing (Table 2). The size of the seeds show that SL (r=0.21) and SW (r=0.27) indicate greater contribution from genitors to progeny with respect to ST (r=0.13) and HW (r=0.09). Phenotypic variation of the traits SL, SL, ST and HW were determined by 11.7, 19.8, 1.5 to 6.5% of additive effects, respectively. Heritability in wide sense (H^2) shows that the largest genetic variations are conditioned to traits SW, ST and HW, however, indicates that the SL is affected by 83.1% of environmental effects. The heritability in restricted sense (h^2) defines actually heritable fraction of genetic variation characteristic. Thus, SL and SW show low heritability (0.11 and 0.19) originating from additive effects, the results obtained for the ST and HW results from the dominance deviations. The obtained genetic parameters in 28 intervarietal hybrids of maize derived from 8 OPVs indicate that the kernel weight is determined by heterosis and dominance effects (Bernini et al., 2012).

Among the constitutional parameters of seeds, AC has progenies relationship with the father-average (r=0.44) superior to SC (r=0.13), pH (r=0.27) and SS (r=0.25). Phenotypic variation of SC, AC, pH and SS are from 3.4, 20.0, 17.2 and 25.2% of additive effects, respectively. Heritability in wide sense indicates greater genetic variation for AC character (H^2=0.50). On the other hand, the SS are influenced by 88.7% of environmental effects. The heritability in restricted sense shows superior additive effects for AC trait (h^2=0.17), pronounceable dominance deviations are expressed for the SC (h^2=0.03).

For phenolic constituents and antioxidant radicals, it is observed that the TP and the DP are higher in relation progenies father-average (r=0.58) and (r=0.60) respectively, associated with TF (r=0.12), TC (r=0.46) and AB (r=0.17). Phenotypic variation of TP, TF, CT, DP and AB are the result of 13.7, 8.6, 48.4, 26.4 and 7.7% of additive effects, respectively. Heritability in wide sense indicates greater genetic variation for TP (H^2=0.62), CT

Table 2. Estimate of genetic parameters, relative progeny with the parent-average (*r*), phenotypic variance (*Vp*), additive variance (*Va*), heritability in wide sense (H^2), heritability in restricted sense (h^2) for the linked traits of dimensions of seeds, bioactive compounds and micronutrients in open pollinated varieties (OPVs) and hybrids maize.

Traits	*r*	*Vp*	*Va*	H^2	h^2
SL[1]	0.215	0.808	0.095	0.169	0.117
SW	0.276	0.419	0.083	0.377	0.199
ST	0.134	0.188	0.003	0.297	0.017
HW	0.092	29.028	1.894	0.252	0.065
SC	0.137	88.223	3.018	0.232	0.034
AC	0.441	0.005	0.001	0.504	0.172
pH	0.272	0.029	0.005	0.053	0.167
SS	0.251	2.297	0.581	0.113	0.253
TP	0.581	11986.934	1648.666	0.620	0.138
TF	0.125	12961.564	1122.750	0.293	0.087
TC	0.461	2296.409	1112.466	0.518	0.484
DP	0.608	43.090	11.393	0.644	0.264
AB	0.179	121.604	9.400	0.204	0.077
Cu	0.676	424.790	0.463	0.810	0.001
Zn	0.274	32.311	0.122	0.261	0.004
Na	0.332	0.607	0.212	0.904	0.349
Mn	0.412	64.835	0.089	0.560	0.001
Fe	0.496	6.595	0.068	0.015	0.010

[1]Seed length (SL); seed width (SW); seed thickness (ST); hundred kernel mass (HW); seed color (SC); seed acid (CA); pH in hydrogenionic potential (pH); soluble solids (SS); total phenols (TP); total flavonoids (TF); total carotenoids (TC); antioxidant potential by DPPH radical (DP); antioxidant potential by ABTS radical (AB); copper (Cu); zinc (Zn); sodium (Na); manganese (Mn); iron (Fe).

(H^2=0.51) and DP (H^2=0.64), however, the TF and AB are influenced by 70.7 and 79.6% of environmental effects. The heritability in restricted sense indicates additive effects of TC (h^2=0.48), higher dominance deviations are assigned to TF (h^2=0.08) and AB (h^2=0.07).

Among the micronutrients, the Cu expressed relation of progenies with father-average higher (r=0.67) than Zn (r=0.27), Na (r=0.33), Mn (r=0.41) and Fe (r=0.49). The phenotypic variation Cu, Zn, Na, Mn and Fe is derived of 0.10, 0.37, 34.92, 0.13 to 1.03% of additive effects. Heritability in wide sense shows greater genetic variation for Cu (H^2=0.81) and Na (H^2=0.90), however, Zn and Fe are affected by 73.9 and 98.5% of environmental effects. The heritability in restricted sense indicates superiority to additive effects of Na (h^2=0.34), however, the greater dominance deviations are established to be Cu (h^2=0.001), Zn (h^2=0.004), Mn (h^2=0.001) and Fe (h^2=0.010).

Determination of the genetic effects of heterosis

The heterosis expresses the increment of the trait through hybrid combination performed (Ramalho et al., 2012). Thus, they carried the fixing intervarietal crosses of the Dente de Ouro Roxo genotype (*P1*), as the male parent is held, by contrast, genotypes BRS Missões (*P2*), Caiano Rajado (P_3), AL 25 (P_4), Bico de Ouro (P_5) and Argentino Branco (P_6) comprised the female parents, where the traits ST, SC, TP, AB and Zn presented positive heterosis for all crossings made (Table 3), that is, the increase of the traits evaluated in relation to the parents average. The P_2 x P_1 crossing provided increased SC and TC with 34.04 and 158.03%, respectively. By combining the genitors P_3 x P_1, the traits ST, TP and DP were increased to 27.02, 39.17 and 53.35% due to the effects of heterosis. The crossing of P_4 x P_1 showed that traits Zn and Fe were increased by 49.77 and 54.10%, however, the crossing of P_5 x P_1 showed increased 67.38% DP radical. However, the hybrid combination of P_6 x P_1 identifies pronounceable effects of Fe and Mn with heterosis of 118.87 and 111.57% in the proportions of these elements in seeds of hybrid of maize.

The results obtained for the six open pollinated varieties (OPVs) and the five intervarietal hybrids of

Table 3. Estimated heterosis percentage (H) for the traits linked to the dimensions of the seeds, bioactive compounds and micronutrients in in hybrids maize.

Traits	Heterosis (%)				
	P_2 x P_1[(2)]	***P_3 x P_1***	***P_4 x P_1***	***P_5 x P_1***	***P_6 x P_1***
SL[(1)]	-14.590	-2.160	-19.480	1.120	-1.800
SW	-3.410	11.060	-3.510	7.730	3.660
ST	17.530	27.020	20.760	3.210	11.060
HW	-24.650	17.300	-13.620	10.540	-12.200
SC	34.040	14.960	15.180	14.500	20.210
AC	-4.680	-11.980	-1.100	2.420	-46.810
pH	7.120	-1.210	-1.040	-1.430	-2.140
SS	37.700	-50.000	37.500	-34.330	-25.000
TP	8.270	39.170	21.630	24.020	8.650
TF	-3.550	-9.350	40.660	29.350	31.470
TC	158.030	10.820	30.490	6.020	-44.960
DP	70.510	53.350	30.510	67.380	-0.240
AB	68.360	9.000	13.800	23.080	8.780
Cu	-99.040	3.860	-4.470	-12.870	-5.370
Zn	32.160	20.550	49.770	40.970	48.170
Na	-44.280	4.350	10.770	-9.090	38.540
Mn	-85.000	-1.560	24.850	14.070	111.570
Fe	-42.680	24.370	54.100	47.200	118.870

[(1)]Seed length (SL); seed width (SW); seed thickness (ST); hundred kernel mass (HW); seed color (SC); seed acid (CA); pH in hydrogenionic potential (pH); soluble solids (SS); total phenols (TP); total flavonoids (TF); total carotenoids (TC); antioxidant potential by DPPH radical (DP); antioxidant potential by ABTS radical (AB); copper (Cu); zinc (Zn); sodium (Na); manganese (Mn); iron (Fe).P_1: Dente de Ouro Roxo; P_2: BRS Missões; P_3: CaianoRajado; P_4: AL25; P_5: Bico de Ouro; P_6: Argentino Branco.

maize allowed evaluating the genetic parameters and gene action mechanisms that influence the phenotypic variation observed. The seed thickness and color, total phenols, antioxidant ABTS radical and zinc are increased by intervarietal heterosis, and are important for the development of maize genotypes in the region of southern Brazil.

Conclusions

Linear high and positive associations are expressed between the seed width and hundred kernel weight, between the antioxidants DPPH and ABTS radical, and through the manganese and zinc. The genetic parameters determine the progenies and the parent-average relationship are superior to the length, width and acidity of seeds, total phenols and copper. The heritability in a wide sense expresses greater than the traits acidity seeds, antioxidant DPPH radical and copper. The heritability in a restricted sense is superior to the traits length, width and acidity of seeds, carotenoids and sodium content. The traits thickness and color of seeds, total phenols, antioxidant ABTS radical and zinc are increased through the effects of intervarietal heterosis in maize. Estimates of genetic parameters obtained can be used in genetic maize breeding programs, in order to obtain biofortified genotypes using bioactive compounds and micronutrients.

Conflict of Interests

The authors have not declared any conflict of interests.

REFERENCES

Abrahão AS, Pereira RGFA, Duarte SMS, Lima AR, Alvarenga JA, Ferreira EB (2010). Bioactive compounds and antioxidant activity of coffee (*Coffea Arabica* L.). Ciênc. Agrotec. 34:414-420.

Andreotti M, Souza E, Crusciol CAC (2001). Morphological components and dry matter production of maize due to application of lime and zinc. Sci. Agric. 58:321-327.

AOAC (2005). Official methods of analysis of the AOAC International. Gaithersburg. United States of American. 100 p.

Argenta GR, Sangoi L, Silva PRF, Rampazzo C, Gracietti LC, Strieder ML, Forsthofer EL, Suhre E (2003). Maize grain yield potential at two environments and five production systems. Sci. Agrar. 4:27-34.

Bernini CS, Paterniani ME (2012). Heterosis parameters estimates in hybrid F_2 populations. Pesqui. Agropecu. Trop. 42:56-62.
Brand-Williams W, Cuvelier M, Berset C (1995). Use of a Free Radical Method to Evaluate Antioxidant Activity. Food Sci. Technol. 28:25-30.
Cardoso WS, Paes MCD, Galvão JCC, Rios AS, Guimarães PEO, Schaffert RE, Borém A (2009). Variability of maize genotypes on the composition of carotenoids in the grains. Pesqui. Agropec. Bras. 44:164-173.
Carvalho FIF, Lorencerti C, Benin G (2004). Estimativas e implicações da correlação no melhoramento vegetal. Pelotas. Brazil.142 p.
Castro MVL, Naves MMV, Oliveira JP, Froes LO (2009). Industrial yield and chemical composition of high quality protein maize as compared to commercial hybrids. Pesqui. Agropecu. Trop. 39:233-242.
Costa NMB, Borém A (2013). Biotechnology in health and nutrition: How DNA can enrich foods. Rio de Janeiro. Brazil. 160 p.
Cruz CD (2013). GENES a software package for analysis in experimental statistics and quantitative genetics. Acta Sci. Agron. 35:271-276.
Cruz CD, Carneiro PCS, Regazzi AJ (2014). Biometric templates applied to breeding. Viçosa. Brazil 668 p.
Estrada BAA, Marco ALV, Yazel NV, Uribe G, Saldívar SOS (2014). Improvement of dietary fiber, ferulic acid and calcium contents in pan bread enriched with nejayote food additive from white maize (*Zea mays*) J. Cereal Sci. 60:264-269.
Falconer DS (1987). Introduction to quantitative genetic. Viçosa. Brazil. 279p.
Gomes PF (2000). Curso de estatística experimental. Piracicaba. Brazil. 477 p.
Junior SM, Caliari M, Vera R, Guazzelli A (2008). Post-harvest conservation of mangaba under refrigeration and modification of storage atmosphere.Pesqui. Agropecu. Trop. 38:78-86.
Kimura M, Kobori CN, Rodriguez-Amaya DB, Nestel P (2007). Screening and HPLC methods for carotenoids in sweet potato, cassava and maize for plant breeding trials. Food Chem. 100:1734-1746.
Kirkby A, Römheld V (2007). Micronutrients in plant physiology: micronutrients in plant physiology: functions, absorption and mobility. Inf. Agro. 118:1-24.
Lopes SJ, Dal Collúcio A, Storck L, Damo HP, Brun B, Santos VJ (2007). Path analysis on maize spikes characteristics related of the hybrid type. Cienc. Rural. 37:1536-1542.
Magalhães PC, Durães FO (2006). Physiology of maize production. Bras. Brazil. 10p.
Menkir A (2008). Genetic variation for grain mineral content in tropical-adapted maize inbred lines. Food Chem. 110:454-464.
Nardino M, Souza VQ, Baretta D, Konflanz VA, Carvalho IR, Follmann DN, Caron BO (2016). Association of secondary traits with yield in maize F1's. Cienc. Rural 46:100-110.
Oliveira DA, Fernandes A (2010). Foliar fertilization and use of flavonoids in maize crop. FAZU Rev. 5:46-50.
Oliveira JP, Chaves LJ, Duarte JB, Brasil EM, Ribeiro KO (2007). Physic quality of the grain in hight quality protein maize populations and their crosses. Pesqui. Agropecu. Trop. 37:233-241.
Paes MCD (2006). Physical, chemical and technological corn grain. Brasília. Brazil. 75:6.
Ramalho M, Santos JB, Pinto CB, Souza EA, Gonçalves FMA, Souza JC (2012). Genetics in agriculture. Lavras. Brazil. 566 p.
Ribeiro LP, Teodoro PE, Corrêa CCG, Oliveira EP, Silva FA, Torres FE (2016). Correlations and genetic parameters in maize hybrids. Biosci. J. 32:48-54.
Rios SA, Alves KR, Costa NMB, Martino HSD (2009). Biofortification: cultivares enriched with micronutrients for breeding. Rev. Ceres. 56:713-718.
Rios SA, Borém A, Guimarães PEO, Paes MCD (2012). Path analysis for carotenoids in maize. Rev. Ceres. 59:368-373.
Rufino MSM, Alves RE, Brito ES, Morais SM, Sampaio CG, Pérez-Jiménez J, Saura-Calixto FD (2007). Determination of the total antioxidant activity in fruits for the capture of free radical ABTS.˙ Bras. Brazil. 128:4.
Sangoi L, Almeida ML, Horn D, Bianchet P, Gracietti MA, Schmitt A, Schweitzer C (2004). Seed size, sowing depth and maize initial growth at two sowing dates.Rev. Bras. de Milho e Sorgo 3:100-110.
Setimela S, Vivek B, Banziger M, Crossa J, Maideni F (2007). Evaluation of early to medium maturing open pollinated maize varieties in SADC region using GGE biplot based on the SREG model, Field Crops Res. 107:161-169.
Shapiro SS, Wilk MB (1965). An Analysis of Variance Test for Normality. Biometrika 52:591-611.
Sikora E, Cieslik E, Leszczynska T, Filipiak Florkiwuacz A, Pisulewski PM (2008). The antioxidant activity of selected cruciferous vegetables subjected to aquathermal processing. Food Chem. 107:55-59.
Silva MLC, Costa RS, Santana AS, Koblitz MGB (2010). Phenolic compounds, carotenoids and antioxidant activity in plant products. Semina: Ciênc. Agrárias. 31:669-682.
Singleton VL, Rossi JAJ (1965). Colorimetry of total phenolics with phosphomolybdic-acid reagents. Am. J.Enol.Viticult. 16:144-158.
Steel RGD, Torrie JH, Dickey DA (1996). Principles and procedures of statistics: a biometrical approach. New York. . United States of American. 672 p.
Tedesco MJ, Gianello C, Bissani CA, Bohnen H, Volkweiss SJ (1995). Soil analysis, plants and other materials. Porto Alegre. Brazil. 174p.
Vazquez GH, Arf O, Sargi BA, Pessoa ACO (2012). Influence the size and shape of the maize seed on plant development and productivity of grains. Biosci. J. 28:16-24.
Wen B, Liu Y, Wang P, Wu T, Zhang S, Shan X, Lu J (2012). Toxic effects of chlortetracycline on maize growth, reactive oxygen species generation and the antioxidant response, J. Environ. Sci. 24:1099-1105.
Zhishen J, Mengcheng T, Jianming W (1999). The determination of flavonoid contents in mulberry and their scavenging effectes on superoxide radicals. Food Chem. 64:555-559.
Zilic S, Janković M, Basić Z, Vančetović J, Maksimović V (2016). Antioxidant activity, phenolic profile, chlorophyll and mineral matter content of corn silk (*Zea mays L*): Comparison with medicinal herbs. J. Cereal Sci. 69:363-370.

academicJournals

Vol. 11(33), pp. 3134-3140, 18 August, 2016
DOI: 10.5897/AJAR2016.11346
Article Number: 02A40E560046
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

The beverage quality of Conilon coffee that is kept in the field after harvesting: Quantifying daily losses

Abraão Carlos Verdin Filho[1], Paulo Sérgio Volpi[1], Wagner Nunes Rodrigues[2]*, Tafarel Victor Colodetti[2], Aldo Luiz Mauri[1], Romário Gava Ferrão[3], Aymbiré Francisco Almeida da Fonseca[4], Maria Amélia Gava Ferrão[4], Lima Deleon Martins[2], Sebastião Vinícius Batista Brinate[2], Marcelo Antonio Tomaz[2], Marcone Comério[1], Saul de Andrade Júnior[2] and Carlos Alexandre Pinheiro[2]

[1]Fazenda Experimental de Marilândia, Instituto Capixaba de Pesquisa, Assistência Técnica e Extensão Rural, Incaper, Marilândia, Espírito Santo, Brazil.
[2]Centro de Ciências Agrárias, Universidade Federal do Espírito Santo, Alto Universitário, 16, Alegre, Espírito Santo, Brazil.
[3]Instituto Capixaba de Pesquisa, Assistência Técnica e Extensão Rural, Rua Afonso Sarlo, 160, Bento Ferreira, Vitória, Espírito Santo, Brazil.
[4]Empresa Brasileira de Pesquisa Agropecuária, Brasília, Instituto Capixaba de Pesquisa, Assistência Técnica e Extensão Rural, Rua Afonso Sarlo, 160, Bento Ferreira, Vitória, Espírito Santo, Brazil.

Received 21 June, 2016; Accepted 26 July, 2016

In search to maintain the coffee quality, the period of time for which the coffee is kept in the plantation after harvest (waiting transportation for the processing site) is one of the several factors that deserves scientific attention. This experiment aimed to identify and quantify losses of beverage quality suffered by Conilon coffee due to the time being kept in the field after harvesting, as an attempt to determine if the permanence of the bags in the plantation after harvest is possible before it causes detrimental effects over characteristics of beverage quality. The experiment followed a completely random design, with 8 treatments and 4 repetitions, using standardized bags of mature fruits of Coffea canephora Pierre ex Froehner that were kept in the plantation field for periods of 0, 1, 2, 3, 4, 6, 8 or 10 days after harvest. Samples from each bag were sent to chemical analyses, and triplicate samples of the processed coffee were sent to three separated sensorial analyses, each one performed by a different laboratory to asset the quality score and traits of the beverage. The results showed that the beverage quality suffers considerable losses due to the time of bags being kept in the field after harvesting. For many quality parameters, the detrimental effects of the permanence at fields start from the very first day, causing reduction of the quality score of the beverage and lowering the classification of the coffee.

Key words: Coffea canephora, harvest, flavor, aroma, time.

INTRODUCTION

Being one of the most popular beverages worldwide, coffee is also the most important agricultural commodity, this product is only less valuable than petrol, regarding the amount of financial value traded (Sunarharum et al., 2014). In addition, the coffee market has been constantly growing in the past years, with an annual growth of 2.4%, with the consumption (149.8 million bags) coming to surpass the production (143.3 million bags) (Ico, 2015). Associated to the increasing consumption of coffee worldwide, there also has been an increase in the search for special coffees, with high quality grains and intensified aroma and flavor, which make possible to obtain superior classes of beverage (Abic, 2010). This fact is related to the coffee consumer increasing the exigence regarding the quality standards, and also to the desire to track the system in which the coffee was produced, requiring environmental respect and social evaluation in the production of the special coffee (Navarini and Rivetti, 2010).

A high quality coffee has differentiated traits related to the chemical composition of the grains, which is affected for several variables along the cultivation and processing, such as the location of the crop, climate conditions, crop management, harvest technique, post-harvest processing, transporting and drying (Franca et al., 2005; Monteiro and Farah, 2012; Sunarharum et al., 2014). The period of time between the harvest of the mature fruits and their transport to the drying site, when the fruit mass is left in bags in the open plantation field, can promote the fermentation of the fruits due to the favorable conditions to enhance the biological processes, which may not only cause losses and damage to the grains, but also interfere in the quality of the beverage of this coffee. The coffee harvest is one of the stages of the production that requires the larger amount of manpower, which becomes limiting in traditional coffee growing regions. Therefore, the transport of the bags to the processing and drying site sometimes cannot be done promptly after harvesting, since some production systems may lack the time or manpower for the immediate process.

However, the traits of beverages made with grains of the specie *Coffea canephora* Pierre ex Froehner have not been focus of many scientific studies, since the coffee quality concept have been connected to *Coffea arabica* L. for many years. Therefore, the magnitude in which environmental factors can deplete its quality are yet to be elucidated, which very little scientific data being available in the subject. One of the best ways to determine and quantify losses caused by unwanted processes over the coffee quality is to perform standard sensorial analyses in the beverage (Monteiro and Trugo, 2005) and chemical analyses in the grains (Silva et al., 2009), which can be used to determine chemical variables with high correlation to sensorial parameters, such as potassium lixiviation and electric conductivity, helping to quantify the quality of the grains (Malta et al., 2005; Martinez et al., 2013; Pinto et al., 2002). The study aimed to identify and quantify losses of beverage quality that the Conilon coffee suffers due to the time of bags being kept in the field after harvesting, as an attempt to determine if the permanence of the bags in the plantation for a time after harvest is possible before it causes detrimental effects over characteristics of beverage quality.

MATERIALS AND METHODS

Characterization of area and coffee plantation

The experiment was conducted in the productive cycle of 2011 to 2012, in an 8 years old standardized plantation of *Coffea canephora* Pierre ex Froehner, with plants spaced 3.0 x 1.0 mm located in the municipality of Marilândia, in Espírito Santo State, Southeast region of Brazil. The altitude of the site is 202 m above sea level, the topography is hilly and the soil is classified as dystrophic Oxisol (Embrapa, 1997). The climate is tropical, classified as Aw (Köppen and Geiger, 1928), typically rainy from November to February; partially dry in March, April and October and dry from May to September, accumulating an average of 1,164 mm of annual rainfall and presenting average annual temperature of 24.2°C (13.9 to 33.5°C).

The plantation was established in 2004, and cultivated until the stabilization of the reproductive cycle, following the recommendations for Conilon coffee in Brazil (Prezotti et al., 2007; Ferrão et al., 2007; Ferrão et al., 2012) to manage nutritional and phytosanitary status of the plants. During the fourth harvest (when the plantation presented over 80% of fully ripe fruits), the fruits were manually collected and stored in raffia bags, which were weighted to create a homogeneous collection of bags of 50 kilograms each. The fruits were processed, roasted and milled after different periods of time to setup the treatments.

Experimental design

The bags were properly identified and randomly selected to be transported at 0, 1, 2, 3, 4, 6, 8 or 10 days after harvest. The experiment followed a completely random design, with 8 treatments (days staying in open field after harvest), and four repetitions. Each experimental plot consisted of one bag of 50 kg of green coffee.

Post-harvest processing and beverage quality

The fruits were transported to cement fields for post-harvest drying, and processed, following the recommendations for coffee in Brazil (Ferrão et al., 2007; Ferrão et al., 2012; Reis et al., 2012). After processing, samples of 50 homogeneous grains were weighed for standardization, immersed in 75 mL of purified water (reverse osmosis) and stored in a laboratory oven at 25°C for 5 h. The electric conductivity of each solution was measured using a digital conductivity meter (Sensoglass, SC-1800, precision: 0.05), and the results were expressed in µS cm^{-1} g^{-1}. The same solutions were used to determine the potassium lixiviation of the samples, using a flame photometer (Digimed, DM-62, precision: 0.001), and the results were expressed in ppm (Kryzyanowski et al., 1991; Malta et al., 2005; Pinheiro et al., 2012).

Homogeneous grain samples from each bag were roasted

*Corresponding author. E-mail: rodrigues@phytotechnics.com.

(Pinhalense, TMC-10) and milled (Pinhalense, MLV-5NA), and sent to three separated sensorial analyses, each one performed by a different laboratory to classified the mean beverage quality (triplicate samples from each bag), determining the sensorial variables (aroma, flavor, finalization, acidity, body, uniformity, balance, clean cup, sweetness, general aspect) of the beverage from each bag.

The cup test was performed by testers registered in Brazil (Ministério da Agricultura, Instrução Normativa Nº8), with three sub-samples of each experimental unit (Brasil, 2003).

Data analyses

The data were subjected to analysis of variance, and according with the presence of significant differences between treatments, the means were studied using regression analyses ($p < 0.05$), verifying the fit to linear models and selecting the models based on the statistical significance of the model, significance of the coefficients and r-square. All statistical tests were performed using the software GENES 5.1. (Cruz, 2013).

RESULTS

The variance analyses showed the existence of differences for most studied variables, the means were similar only for the one organoleptic characteristic of the beverage, which was the body. The coefficients of variation were under 10.89%, with this higher coefficient being observed for the clean cup test. Regarding the parameters of beverage quality, regression analyses presented fit to linear models of first degree for: aroma, flavor, finalization, balance, clean cup, sweetness and general score; and second degree for: acidity and uniformity (Figure 1). For the total score of the beverage quality, a fit to a first degree linear model was observed, while a second degree model was adequate to describe the behavior of the electric conductivity and lixiviation of potassium (Figure 2).

DISCUSSION

The aroma of the beverage is one of the major traits of coffee that is perceived by consumers as a critical characteristic to determine their liking and, as shown in Figure 1a, a linear loss in the aroma happened due to the time of the green coffee being kept in the field after harvesting. Fisk et al. (2012) affirms that different aroma profiles in coffee beverages can be originated from several sources along the cultivation processes, such as post-harvest processing type and aging before roasting, as well as seasonal variation and climatic conditions (Da Silva et al., 2005) and geographical locations (Risticevic et al., 2008). Regardless of presenting low aromatic traits, green beans contain several chemical precursors that will significantly contribute to determine the concentration of the complex compounds mixture that generate the aroma profile of the beverage, and the concentration of these chemical precursors is dependent on the origin and treatment of the coffee beans (Fisk et al., 2012).

According to Sunarharum et al. (2014), flavor is arguably the most important trait of the coffee beverage for the consumer and it is result of a series of complex chemical, biological and physical influences. The same authors describe that coffee flavor is generated still in the coffee plant, during the development of the fruits, and goes on throughout the harvest, processing and preparation techniques. Exposing the fruits to environmental conditions, such as humid conditions (Ahmad et al., 2003) and presence of microorganisms, during extended periods of time can cause the development of different flavor traits. Undesirable flavor traits linearly developed in this study (Figure 1b) due to the increase in the time of exposure to environmental conditions of the plantation after harvest. A high quality coffee should present enjoyable finalization, with adequate residual effect. The increase in the time kept in the plantation caused the finalization score to decrease linearly, losing near 0.07 score per day (Figure 1c). The influence of the plantation environment probably promoted biochemical alterations in the fruits that were detrimental to the maintenance of the aroma and flavor in the beverage which, in association with the less intense flavor and aroma, caused the reduction in the score for finalization of the beverage. The acidity presented a slight increase followed by the decrease of the score after the third day being kept at the plantation (Figure 1d). This short term increase in the acidity score along the first 3 days should not be explored, since the acidity is very important to define the coffee quality, but only in combination with sweetness, bitterness and aroma profile (Sunarharum et al., 2014), which presented daily decreased.

According to Bicho et al. (2013), with the increasing consumption worldwide, the interest of industry and scientists in the sensory properties has been growing. Recently, different sensory traits have been used to describe the attributes of the coffee beverage, including body, which is related to the tactile impression of texture, viscosity and mouthfeel of the beverage. This sensory trait was not affected by extending the period of time kept in the plantation (Figure 1e), but since the quality of gourmet coffees depends on the combination of flavor, body and aroma (Mori et al., 2003), the depreciation of the other beverage traits still resulted in beverage of lower qualities. The uniformity score is higher when the coffee is moved to processing right after harvesting, decreasing with time (Figure 1f), but presenting increase after 5 days. The uniformity of the mass with the longer periods of time is not passible of exploration, as the beverage becomes again more homogeneous, but uniform with low quality standards, consistently keeps the undesirable flavor in different cups of the sample tasted.

Regarding the balance, the linear decrease (Figure 1 g) observed with the increase of the time kept in the

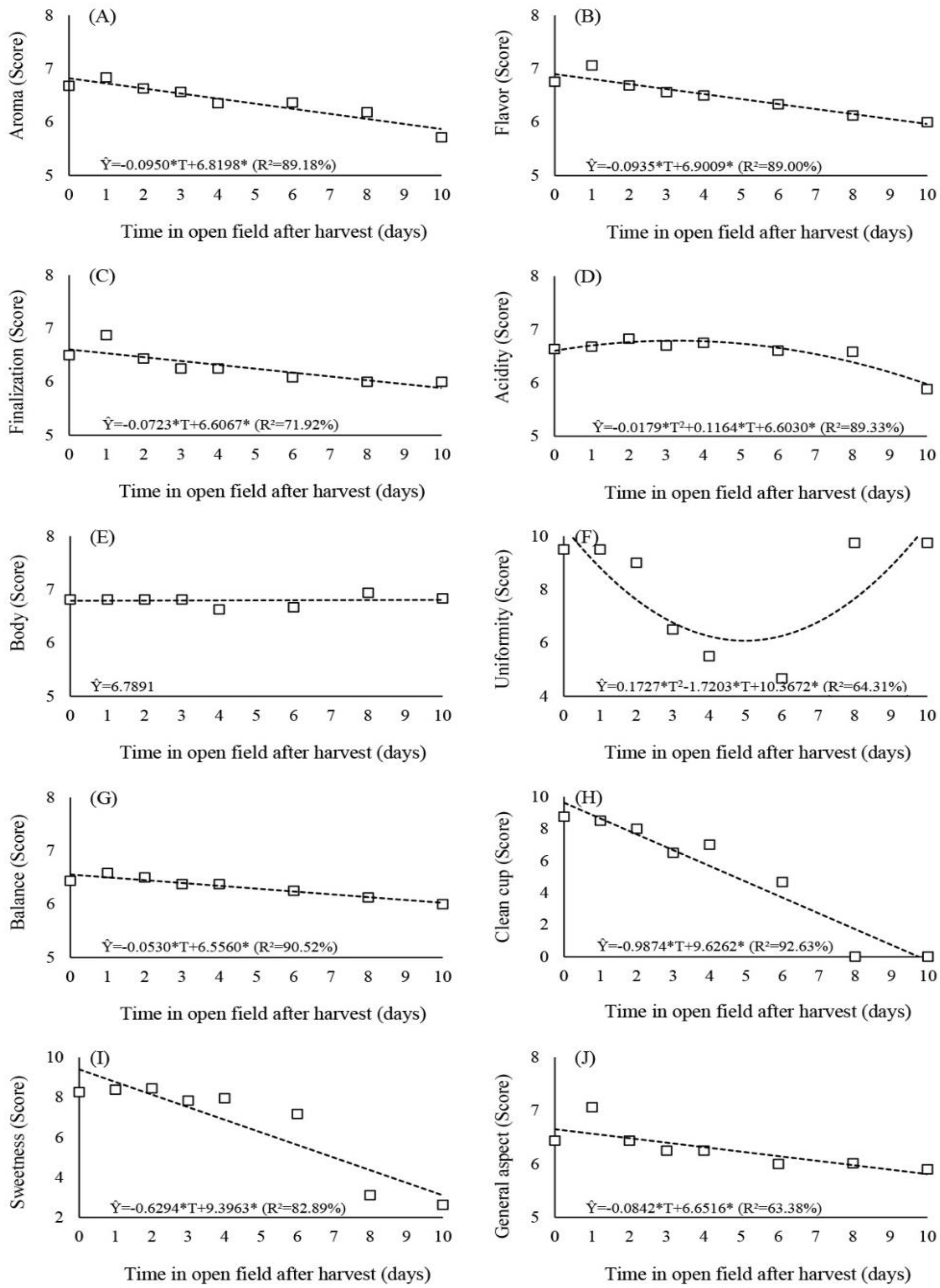

Figure 1. Parameters of beverage quality of Conilon coffee as function of the time being kept in the field after harvesting: aroma (A), flavor (B), finalization (C), acidity (D), body (E), uniformity (F), balance (G), clean cup (H), sweetness (I), general aspect (J) (Marilândia, Espírito Santo, Brazil, 2011-2012).

plantation shows the continued degradation of the quality, the decrease of balance is undesirable as this trait determines the pleasant sensation to the taste during the consumption and after tasting (Mori et al., 2003). Clean cup is related to the lack of negative interferences from first ingestion to final aftertaste, being commonly referred to as the "transparency" of the cup (Scaa, 2015a). This attribute presented a sharp decrease with the influence of the environmental factors over the coffee in the plantation, with losses already being found from the first

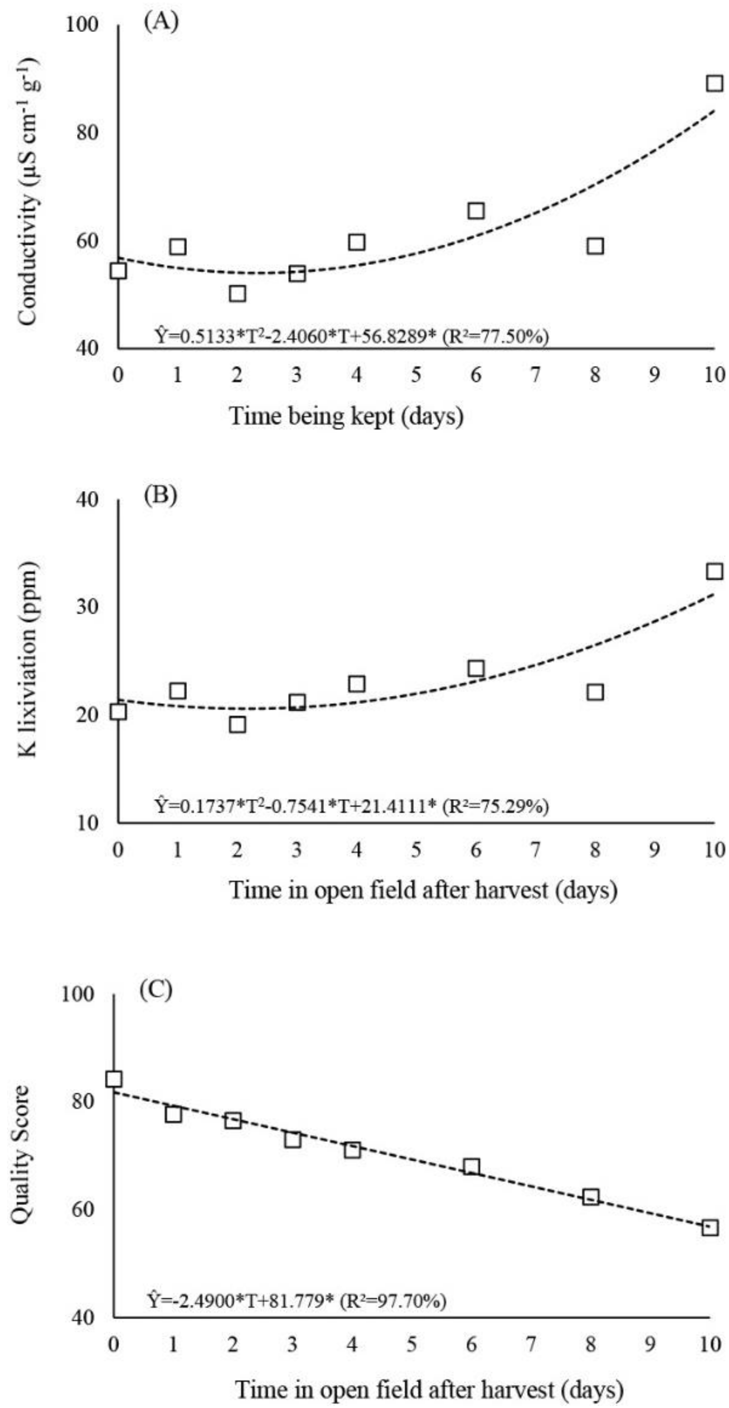

Figure 2. Characteristics of quality of Conilon coffee as function of the time being kept in the field after harvesting: electric conductivity (A), lixiviation of potassium (B) and score of the beverage (C) (Marilândia, Espírito Santo, Brazil, 2011-2012).

day being kept at the field (Figure 1h). A severe decline in sweetness was observed with the time being left at plantation (Figure 1i), it is possible that the slightly increase in the acidity caused by the treatments contributed to the initial decrease in the sweetness, as these attributes of the beverage often inversely correlated (Sunarharum et al., 2014). The sharp decrease continues even after the acidity past the minimum point and start increasing with the time kept in the plantation, this behavior may be related to the alteration of the compounds with the time which may be able to change the correlation between these traits as the coffee mass is kept under environmental conditions.

The general aspect or overall scoring aspect represents the integrated rating of the sample as perceived by specific panelists. The treatments caused decrease in this trait (Figure 1j), therefore, the coffee being kept for longer periods of time at plantation becomes less likely to meet the expectations, losing its capacity to express its character and to reflect qualities of original flavor (Scaa, 2015a). The chemical analyses of electric conductivity and lixiviation of potassium both presented similar behavior (Figure 2a and b), with increasing values as the time being kept at the plantation increased. These variables are correlated negatively to the beverage quality or the presence of defective grains (Malta et al., 2005; Prete, 1992; Romero et al., 2003). The final scoring of the coffee is calculated by first summing the individual scores given for each of the primary attributes and subtracting the defects. The total score can then be used to determine the quality classification of the coffee (Scaa, 2015a). The time being kept in the plantation alone caused the loss of classification from a specialty coffee of very good quality to a lower quality and not specialty class coffee (Figure 2c). The standards for scoring Robusta coffee proposed by Scaa (2015b) would indicate, considering a relative proportional loss, a decrease from the classification Fine coffee to a usual coffee from the time being kept in the plantation alone.

Conclusion

Chemical changes promote noticeable alterations in the coffee grains from the first day being kept in open field. The beverage quality of Conilon coffee suffers considerable losses due to the time of bags being kept in the field after harvesting. For many primary parameters of quality, the detrimental effects of the permanence at fields start from the very first day, causing reduction of the quality score of the beverage and lowering the classification of the coffee. Therefore, it is recommended to transport the harvested coffee out of fields as soon as possible to reduce the detrimental effects over the beverage quality.

Conflicts of Interests

The authors have not declared any conflicts of interests.

REFERENCES

Abic (Associação Brasileira da Indústria de Café) (2010). Tendências de consumo de café. Access at: 20 December 2015.

Ahmad R, Tharappan B, Bongirwar DR (2003). Impact of gamma irradiation on the monsooning of coffee beans. J. Stored. Prod. Res. 39(2):149-157.

Bicho NC, Leitão AE, Ramalho JC, Alvarenga NB, Lidon FC (2013). Impact of roasting time on the sensory profile of Arabica and Robusta coffee. Ecol. Food. Nutr. 52(2):163-177.

Brasil (2003). Ministério da Agricultura, Pecuária e Abastecimento. Instrução normativa, n. 8, de 11 de junho de 2003. Brasília, Mapa 11p.

Cruz CD (2013). GENES: a software package for analysis in experimental statistics and quantitative genetics. Acta Sci. Agron. 35(1):271-276.

Da Silva EA, Mazzafera P, Brunini O, Sakai E, Arruda FB, Mattoso LHC, Carvalho CRL, Pires RCM (2005). The influence of water management and environmental conditions on the chemical composition and beverage quality of coffee beans. Braz. J. Plant Physiol. 17(2):229-238.

Embrapa (Empresa Brasileira de Pesquisa Agropecuária) (1997). Manual de métodos de análises de solo. Rio de Janeiro: Mapa 212 p.

Ferrão RG, Fonseca AFA, Bragança SM, Ferrão MAG, DeMuner LH (2007). Café Conilon. Vitória, Incaper, 2007. 702p.

Ferrão RG, Fonseca AFA, Ferrão MAG, Verdin Filho AC, Volpi PS, DeMuner LH, Prezotti LC, Ventura JA, Martins DS, Mauri AL, Marques EMG, Zucateli F (2012). Café Conilon: técnicas de produção com variedades melhoradas. (4th ed.). Vitória, Incaper 75p.

Fisk ID, Kettle A, Hofmeister S, Virdie A, Kenny JS (2012). Discrimination of roast and ground coffee aroma. Flavour. 1(14):1-9.

Franca AS, Oliveira LS, Mendonça JCF, Silva XA (2005). Physical and chemical attributes of defective crude and roasted coffee beans. Food Chem. 90(1-2):89-94.

Ico (International Coffee Organization) (2015). Infographics on the global coffee trade. London, Ico. Access at: 11 December 2015.

Köppen W, Geiger R (1928). Klimate der Erde. Gotha: Verlag Justus Perthes.

Kryzyanowski FC, França Neto JB, Henning AA (1991). Relatos dos testes de vigor disponíveis as grandes culturas. Inform. Abrates 1:15-50.

Malta MR, Pereira RGFA, Chagas SJR (2005). Condutividade elétrica e lixiviação de potássio do exsudato de grãos de café: alguns fatores que podem influenciar essas avaliações. Ciênc. agrotec. 29(5):1015-1020.

Martinez HEP, Poltronieri Y, Farah A, Perrone D (2013). Zinc supplementation, production and quality of coffee beans. Rev. Ceres. 60(2):293-299.

Monteiro MC, Farah A (2012). Chlorogenic acids in Brazilian Coffea Arabica cultivars from various consecutive crops. Food Chem. 134(1):611-614.

Monteiro MC, Trugo LC (2005). Determinação de compostos bioativos em amostras comerciais de café torrado. Quím. Nova. 28(4):637-641.

Mori EEM, Bragagnolo N, Morgano MA, Anjos VDA, Yotsuyanagi K, Faria EV, Iyomasa JM (2003). Brazil coffee growing regions and quality of natural, pulped natural and washed coffees. Food Foods Ingredients J. Jpn. Food 208(1):416-423.

Navarini L, Rivetti D (2010). Water quality for Espresso coffee. Food Chem. 122(2):424-428.

Pinheiro PF, Costa AV, Queiroz VT, Alvarenga LM, Partelli FL (2012). Qualidade do café 'conilon' sob diferentes formas de secagem. Enc. Biosfera 8(15):1481-1489.

Pinto NAVD, Fernandes SM, Giranda RN, Pereira RGFA, Carvalho VD (2002). Avaliação de componentes químicos de padrões de bebida para preparo do café expresso. Ciênc. Agrotec. 26(4):826-829.

Prete CEC (1992). Condutividade elétrica do exsudato de grãos de café (*Coffea arabica* L.) e sua relação com a qualidade da bebida. Piracicaba, Escola Superior de Agricultura Luiz de Queiroz. 134p.

Prezotti LC, Gomes JA, Dadalto GG, Oliveira JA (2007). Manual de recomendação de calagem e adubação para o Estado do Espírito Santo. (5th ed.). Vitória, Seea/Incaper/Cedagro 301 p.

Reis PR, Cunha RL, Carvalho GR (2012). Café arábica: pós-colheita ao consumo. Lavras, Epamig. 896 p.

Risticevic S, Carasek E, Pawliszyn J (2008). Headspace solid-phase microextraction-gas chromatographic-time-of-flight mass spectrometric methodology for geographical origin verification of coffee. Anal. Chim. Acta 617(1-2):72-84.

Romero JCP, Romero JP, Gomes FP (2003). Condutividade elétrica

(CE) do exsudato de grãos de *Coffea arabica* em 18 cultivares analisados no período de 1993 a 2002. R. Agricultura 78(3):293-302.

Scaa (Specialty Coffee Association of America) (2015a). Cupping protocols. Access at: 01 December 2015. https://www.scaa.org/?page=resources&d=cupping-protocols

Scaa (Specialty Coffee Association of America) (2015b). Fine Robusta standards and protocols. Access at: 17 July 2016. https://www.coffeestrategies.com/wp-content/uploads/2015/04/compiled-standards-distribute1.1.pdf

Silva MC, Castro HAO, Farnezi MMM, Pinto NAVD, Silva EB (2009). Caracterização química e sensorial de cafés da chapada de Minas, visando determinar a qualidade final do café de alguns municípios produtores. Ciênc. Agrotec. 33(s):1782-1787.

Sunarharum WB, Williams DJ, Smyth HE (2014). Complexity of coffee flavor: a compositional and sensory perspective. Food Res. Int. 62:315-325.

academicJournals

Vol. 11(33), pp. 3141-3153, 18 August, 2016
DOI: 10.5897/AJAR2016.11147
Article Number: 719DB7960051
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Assessing the performance of innovation platforms in crop-livestock agro-ecosystems in the Volta basin

Augustine A. Ayantunde[1]*, Kees Swaans[2], Hubert Some[3] and Pamela Pali[4]

[1]International Livestock Research Institute, 01BP 1496 Ouagadougou, Burkina Faso.
[2]International Livestock Research Institute, Addis Ababa, Ethiopia.
[3]Netherlands Development Organization (SNV), Ouagadougou, Burkina Faso.
[4]International Institute of Tropical Agriculture (IITA), P.O. Box 7878, Kampala, Uganda.

Received 22 April, 2016; Accepted 1 August, 2016

To enhance integrated rainwater management in crop-livestock systems in the Volta basin of Burkina Faso, innovation platforms (IP) comprising of multiple stakeholders were established in the districts of Koubri and Ouahigouya. Quarterly IP meetings were organized to collectively identify and prioritize constraints and opportunities, and to design and implement strategies to address them. IP represents an example of putting the agricultural innovations systems' perspective into practice. Several studies have evaluated the performance of IPs, but these are often based on external (mainly qualitative) assessments during mid-term and/or end evaluation. In this study we are interested in how key processes develop over time and how this is perceived by participants themselves, since this determines the participation and commitment of stakeholders and hence the success of the IP. To ensure adequate documentation of IP processes and activities, several monitoring and evaluation tools were developed. This paper focuses on the assessment of the IP performance in terms of consistency of participation across meetings and stakeholder groups, relevance of identified issues, information exchange, conflict resolution, participation in decision making, facilitation, and perceived benefits. For all the indicators used to assess the IP, the mean scores tended to increase with the lifespan of the IPs. This reaffirms that the IP is perceived as valuable by its members as a way to enhance agricultural development. At the same time though an IP is not a "quick-win", but takes time to mature for it to become fully functional and achieve desired outcomes.

Key words: Agricultural development, innovation systems, crop-livestock systems, social learning, Volta basin.

INTRODUCTION

There is a general consensus that a linear approach to agricultural research and development, has had limited success in sub-Saharan Africa (Leeuwis and van de Ban, 2004; Adekunle et al., 2012; Hounkonnou et al., 2012). For example, many technologies have been generated through agricultural research in sub-Saharan Africa and transferred by extension workers to farmers, but their adoption and impact on productivity and livelihoods of

*Corresponding author. E-mail: a.ayantunde@cgiar.org.

rural households have been sub-optimal (Adekunle et al., 2012). In their review on intensification of farming systems in sub-Saharan Africa, Pretty et al. (2011) rightly observed that productivity increases though improved technologies does not necessarily translate into improvement in livelihood of the rural poor without proper consideration of socio-economic, policy and institutional contexts (Hounkonnou et al., 2012). Therefore, to enhance technology uptake and sustainable intensification of agricultural systems, it is essential to include all relevant stakeholders in the process of agricultural innovation.

To address agricultural innovation through participation of multiple stakeholders, the Forum for Agricultural Research in Africa (FARA) has promoted the Integrated Agricultural Research for Development (IAR4D) approach based on an innovation systems framework (Adekunle et al., 2012). The agricultural innovation systems' framework emphasizes the collective nature of innovation and stresses that innovation is a co-evolutionary process, resulting from alignment of technical, social, institutional and organizational dimensions (Lundvall, 2011). Increasingly, the innovation systems framework is also applied to commodity value chains, which can be seen as systems comprising different types of actors in which knowledge and/or research products with purchased and farm- or household-provided inputs are used and developed in natural resource based production, and are marketed and processed for sale or consumed (Adekunle et al., 2012; Van Rooyen and Homann-Kee Tui, 2009).

Operationalization of the IAR4D approach is often through a multi-stakeholder platform referred to as innovation platform (IP). In the context of commodity value chains, the IP[1] is a dynamic and fluid assembly of actors along the chain to support action learning, and actors' linkages, provide opportunities to generate innovation, and strategies for scaling up and out (Pali and Swaans, 2013). An IP facilitates research and learning that not only generates new knowledge, products or technologies, but also ensures the use of research products (Adekunle et al., 2012). Generally, an IP is a mechanism to enhance communication and innovation capacity among mutually dependent actors, by improving interactions, coordination, and coherence among all actors to facilitate learning and contribute to production and use of knowledge (Pali and Swaans, 2013).

The Integrated Agricultural Research for Development (IAR4D) approach is central to the design, testing and evaluation of agricultural technologies and for scaling up and out of promising technologies as it enhances the engagement of relevant stakeholders in participatory action research (Adekunle et al., 2012). The necessity of engaging other stakeholders apart from researchers is driven by the realization that innovation does not arise only from a simple process of transferring knowledge from research to end-users but necessitates a process of interaction and learning from diverse sources whereby the agricultural research organizations are part of a much larger constellation of knowledge producers. The emphasis of the collective nature of innovation is the core of the agricultural innovation systems framework (Lundvall, 2011; Hounkonnou et al., 2012; Kilelu et al., 2013; Schut et al., 2015). The innovation systems framework stresses that innovation occurs through the collective interplay among many actors including farmers, researchers, extension officers, service providers and development organizations. It has to be emphasized that innovations are not just about technology but also include social and institutional change (Leeuwis and van de Ban, 2004).

One of the challenges of IP is systematic monitoring and evaluation (M&E) of its key processes and outcomes (Klerkx et al., 2012). To address this challenge, a host of approaches have been developed to monitor and evaluate the activities of IPs using both quantitative and qualitative methods (Pali and Swaans, 2013; Cadilhon, 2013). Unlike result-oriented monitoring and evaluation (M&E) which is often applied in evaluating results against pre-defined objectives and indicators, the approach to monitor and evaluate IP activities should be more flexible, participatory, and reflexive as IPs interact and affect the environment within which they operate. In this study, we applied a reflexive monitoring approach, using broadly defined qualitative and quantitative parameters based on the IP literature to assess the performance of two IPs in Burkina Faso. Table 1 provides information on key parameters, tools, and indicators.

The CPWF Volta Basin Development challenge project on integrated management of rainwater in crop-livestock systems, took an overarching IAR4D approach and established IPs that brought together relevant stakeholders to come to more effective and sustainable water management practices, leading to improved Value Chain (VC) performance and farmer livelihoods. While the inclusion of relevant stakeholders in agricultural research for development is not questioned, there has been discussion to what extent IPs are useful and effective in boosting African agriculture. Several studies have tried to shed more light on the performance of IPs by studying and revealing key processes and outcomes, but these studies are often based on external (mainly qualitative) assessments during mid-term and end of project evaluation (Schut et al., 2015; Swaans et al., 2014; Adekunle et al., 2012). For the purpose of this study, we were more interested in tracking the development of IP processes over time and how these are perceived by participants themselves, since this may explain participation and commitment of stakeholders and eventually the success of the IP. The objective of this paper is therefore to assess the performance of the IP in terms of its key processes in two project sites in Burkina

[1] In this paper, an IP is sometimes referred to as multi-stakeholder platform. Other authors have referred to IPs as "innovation configurations" (Engel 1995), "innovation networks (Klerkx et al. 2012) and "learning alliances" (Boogaard et al. 2013; Cullen et al. 2014).

Table 1. Analytical framework for evaluation of Innovation Platform (IP) functioning processes in Koubri and Ouahigouya, Burkina Faso.

Key process	Tool	Indicator
Participation	Actor's register: This was administered at every IP meeting to monitor general trend in participation. Meeting report: This provided an overview of participation and agenda of each meeting and the plan for next meeting.	Number of participant at the IP meeting Number of different actor's groups in attendance
Relevance of identified issues	IP assessment tool: This is a semi-structured questionnaire administered after the IP meeting asking the members to assess IP functioning. Activity report: The report captured field activities by the IP members as agreed at the meeting and served as input for IP meeting.	Score IP assessmentNumber and type of activities proposed at the IP meeting which were carried out
Information exchange	IP assessment tool Report of IP activities: The report captured report by the IP members on their interactions with different actors between the IP meetings.	Score from IP assessment Feedback on the number of new contacts through the IPs
Understanding of IP issues	IP assessment tool. Activity report: This captured field activities by the IP members as agreed at the meeting	Score from IP assessment Number and type of activities proposed at the IP meetings which were carried out
Conflict resolution	IP assessment tool Activity report	Score from IP assessment Number and type of activities jointly implemented
Decision making	IP assessment tool Activity report	Score from IP assessment Proportion of IP members who participated in identified activities
Facilitation	IP assessment tool Activity report	Score from IP assessment Feedback from IP members on the quality of facilitation
Benefits and achievements	IP assessment tool Activity report Report of producer groups assessment	Score from IP assessment Number and type of activities proposed at the IP which were carried out

Faso over time based on stakeholders' own views.

METHODOLOGY

The study sites included Ouahigouya and Koubri districts in the Volta basin of Burkina Faso (Table 2). Crop-livestock systems dominate the farming systems in the study sites. Agricultural activities are heavily dependent on rainfall, which accounted for the sources of livelihood of more than 80% of the populations (Douxchamps et al., 2014). The dominant crops cultivated in the study areas were maize, sorghum, millet, cowpea and groundnut. Rice is often cultivated in the floodplain and around the small reservoirs. Koubri has a very good market access as it is very close to Ouagadougou, the capital of Burkina Faso. In each district, four communities were selected for the project activities on integrated management of rainwater in crop-livestock systems. Based on the findings from participatory rural appraisal and value chains analysis, key actors along crop-livestock value chains were identified and brought together to set up four innovation platforms (two in each district) involving stakeholders from the project communities in July 2011. However, the number of IPs was later reduced to two due to overlap in stakeholders involved and for better facilitation of the IPs. At the participatory rapid appraisal in each community, the farmers and livestock keepers were asked to select 2 to 4 focal persons to participate at the IP including at least a woman. Other actor's groups (trader, processor, technical services, researchers, development agencies, and credit agency) in the IP also nominated between 2 and 4 focal persons to participate at the quarterly IP meeting. These focal persons were charged with responsibility of providing feedbacks from the meetings to their members as well as lead the implementation of IP activities agreed at the meetings.

At the first IP meeting, opportunities and constraints to rainwater management in crop-livestock systems from the baseline studies conducted by the project were discussed and prioritized as well as strategies to improve identified crop and livestock value chains. The promising value chains identified from the value chain analysis by the project were discussed at the IP meeting and prioritized for crops and livestock although the IPs initially had a strong production focus and only at a later stage focused more on market access. The prioritized value chains were sorghum, maize, and cowpea for crops while sheep and goat value chains were selected for livestock. Key actors that participated at the inception IP meeting included farmers (both male and female), traders, livestock

Table 2. Main features of the project sites (districts) in Burkina Faso.

District	Location	Annual rainfall	Major soil types	Major livestock species	Major crops	Market access
Koubri district	Kadiogo Province of Burkina Faso 12°11'N and 1°24'W	800 mm	Lixisols	Sheep, goat, cattle	Sorghum, millet, maize, cowpea, groundnut	Very good
Ouahigouya district	Yatenga Province of Burkina Faso 13°34'N and 2°25'W	600 mm	Lixisols with gravel overlying	Cattle, sheep, goat	Sorghum, millet, cowpea	Moderate

Table 3a. Key issues discussed at the IP meetings and resulting activities carried out in Ouahigouya, Burkina Faso from March 2012 to June 2013.

Timeline	Key issue discussed	Resulting activities carried out before the next IP meeting
Mar 2012*	Feedback on cropping season of 2011; animal health and vaccination; soil fertility; discussion on action research protocol	Contact with Department of Livestock Services to arrange for vaccination of cattle, construction of vaccination park, mobilization for vaccination campaign, vaccination of cattle against Contagious Bovine Pleuro Pneumonia (CBPP) and training on compost making
Jun 2012	Access to improved seed for 2012 cropping season, implementation of integrated soil-water-crop and livestock trials, access to credit, discussion on national texts for environmental protection, rehabilitation of degraded land	Procurement of improved seed (sorghum, millet and cowpea) from national agricultural research institute (INERA) and distribution among farmers, formation of "Kolgweogo" (in Moore meaning: "We are near the forest") association to combat against deforestation, meeting of producers with credit agency "Caisse Populaire" on conditions to access credit
Sep 2012	Group marketing of agricultural produce, animal theft, capacity building, postharvest management of crop produce, plan for off-season agricultural activities mainly vegetable production	Construction of enclosures in each community where the animals can stay in the night to reduce theft, formation of surveillance committee at village level to stop animal theft, contact with regional water department for a well for vegetable production in one of the project communities (Kourra Bagre)
Dec 2012	Marketing of agricultural produce, postharvest management, soil fertility management, plan for IP activities in 2013, animal fattening	Training on half-moon water harvesting technique, collection of stones for stone bunding, building of storage shed for onions and group marketing of onions, training on conservation and use of crop residues as animal feed
Mar 2013	Review of IP activities in 2012, access to credit, commercialization of agricultural production, capacity building	Organized training on group marketing and commercialization (in March 2013)
Jun 2013	Registration of IP, evaluation of IP achievements since inception, IP sustainability	Group work by IP members to score IP achievements based on a set of criteria developed by the project team, contact with local government authority on procedures for registration of IP

*This was the third meeting; the rules and key issues were identified and prioritized during the first two meetings, based on a value chain analysis and baseline.

keepers, input suppliers, technical agents, researchers, and non-governmental organizations involved in microcredit. Subsequent IP meetings held quarterly focused on different issues jointly identified by the facilitators and IP members which included soil and water conservation techniques, access to credit, access to technical services, training in marketing of agricultural produce, and monitoring and evaluation of IP processes and outcomes.

To ensure adequate documentation of IP processes and activities, and for evaluation of the performance of the IPs, monitoring and evaluation tools were developed comprising of register of actors, IP meeting and activity report, and members' assessment of the IP. These monitoring and evaluation tools were administered at each IP meeting by an enumerator who was proficient in the local language of the IP members starting from 6 months after the establishment of the IP in Koubri and Ouahigouya till the last meeting before project end (March 2012 up till June 2013). The key issues discussed at the IP meetings and the resulting activities carried out before the next IP meeting are presented in Table 3a and b.

To assess the functioning of both IPs in terms of activities carried out, processes and outputs, a semi-structured questionnaire was administered at the end of every IP meeting from March 2012 to June 2013. Members representing the actors' groups at the IP were asked to score individually the IP functioning on a scale of 1 (minimum) to 5 (maximum) on a number of parameters for analyzing IP performance. The parameters included understanding and relevance of the IP goals and issues addressed, extent of participation in decision making at the IP, extent of information flow

Table 3b. Key issues discussed at the IP meetings and resulting activities carried out in Koubri, Burkina Faso from March 2012 to June 2013.

Timeline	Key issue discussed	Resulting activities carried out before the next IP meeting
Mar 2012	Protocol for participatory action research, access to agricultural inputs (seed and fertilizer), soil fertility, animal vaccination	Contact with Department of Livestock Services to arrange for vaccination of cattle, mobilization for vaccination campaign, vaccination of cattle against Contagious Bovine Pleuro Pneumonia (CBPP), training on compost making and stone bunding
Jun 2012	Access to credit, formal registration of IP, fodder production, soil fertility, improved agronomic practices	Distribution of improved seed (sorghum, millet and cowpea) from national agricultural research institute (INERA) and distribution among farmers, contact with credit agency “Caisse Populaire” on conditions to access credit, implementation of integrated soil-water-crop and livestock trials, exchange visit between 2 communities involved in the action research, meeting between producers and agricultural agent on improved agronomic practices, contact with local government authorities on IP registration
Sep 2012	Follow up on access to credit, conservation of crop residue, planning of off-season agricultural activities, mainly vegetable production, postharvest management of crop produce, sheep fattening	Training on conservation and use of crop residues as animal feed, land preparation for vegetable production, formation of producers’ association to access credit
Dec 2012	Group marketing and commercialization of agricultural production, improved feeding strategies, plan for IP activities in 2013, sheep fattening, capacity building	Training on group marketing and commercialization (February 2013), training on conservation and use of crop residues and profitable sheep fattening
Mar 2013	Review of IP activities in 2012, access to credit, diversification of agricultural production	Organized training on group marketing and commercialization,
Jun 2013	Evaluation of IP achievements since inception	Group work by IP members to score IP achievements based on a set of criteria developed by the project team

and sharing among the actors, conflict resolution within the IP, facilitation of IPs, and perceived benefits of IP activities and achievement of the IP goals. To elaborate on the IP members’ perception on the benefits of their participation at the meetings, we asked two types of producers (crop farmers (crop-dominated livelihood strategy) and livestock keepers (livestock-dominated livelihood strategy, mainly agro-pastoralists) at the last IP meeting of June 2013 on what they perceived as gains on a scale of 0 (none) to 10 (highly satisfactory) based on the criteria determined by the project team. Data collected included gender of the participants and their groups along the value chains namely producer, trader, processor, credit agency, technical service, researcher and development practitioner (mainly NGO).

The IP meetings were facilitated by the Netherlands Development Organization (SNV, Burkina Faso) and Fédération Nationale des Groupements Naam (FNGN, a local NGO in Burkina Faso), with backstopping from the International Livestock Research Institute (ILRI). SNV and FNGN had extended and strong experience in facilitation of multi-stakeholder platforms, and were selected to facilitate the IPs to avoid domination by research organizations (Boogaard et al., 2013).

Data analysis was performed with SAS (Statistical Analysis System Institute, 1987) using the Means Procedures for summary statistics and General Linear Model (GLM) procedures for variance and regression analyses for the data on members’ assessment of IP functioning. For analysis of variance and regression model, response (dependent) variables were the six indicators of IP performance namely understanding and relevance of issues addressed by the IP, participation in decision making, information flow and sharing among actors, conflict resolution, quality of facilitation of IP and perceived benefit of IP. The independent variables were IP location (Koubri and Ouahigouya), period of IP meeting, actors’ group and gender. For the regression analysis, the independent variables were considered as binary categorical variables with value of either 0 or 1. Unless otherwise specified, the level of significance was declared at $p < 0.05$.

RESULTS AND DISCUSSION

The assessment of IP performance is described and discussed in three parts: Participation of stakeholders and key issues identified the perception of key processes and benefits by stakeholders, and achievements as perceived by crop and livestock farmers.

Participation and major activities carried out by the innovation platforms

The number of participants at the IP meetings from March 2012 to June 2013 varied from 24 to 35 in Ouahigouya and from 30 to 42 in Koubri (Figure 1). The

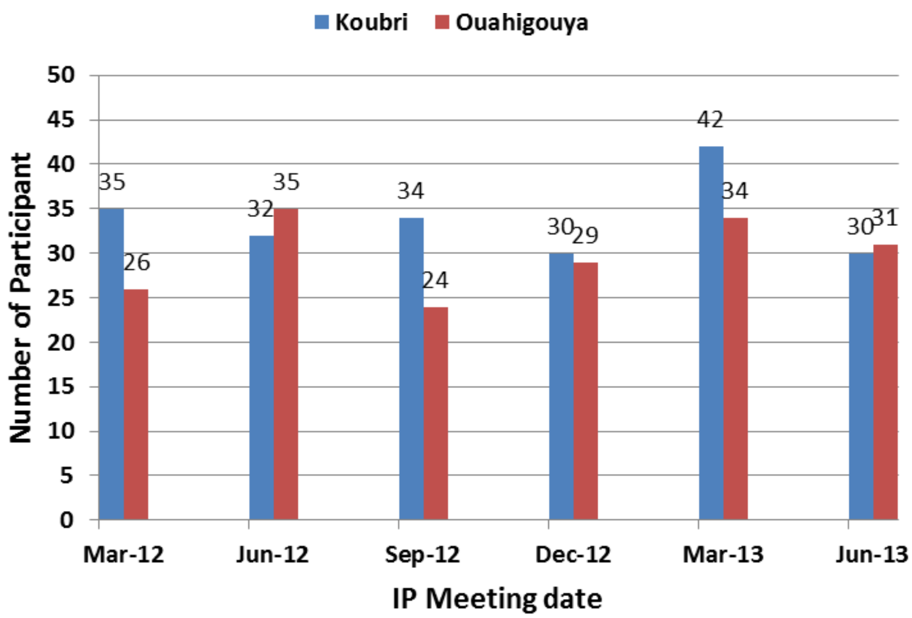

Figure 1. Number of participants at different innovation platform meetings in Koubri and Ouahigouya, Burkina Faso.

highest attendance was recorded in Koubri directly following the training on commercialization and marketing of agricultural produce in February 2013 which attracted many actors (including farmers, State technical agents and development practitioners) (March, 2013). Given the diversity of IP actors, stakeholders are only likely to participate in IP meetings when there are issues of shared or common interest (Boogaard et al., 2013). As a result of domination of farmers in the IPs in the two study sites, production issues tended to dominate the agenda as shown in Table 3a and b but these issues often complied well with the mandate or objectives of other stakeholders in the IP. Though production issues are the most obvious and priority constraint; however, more emphasis was paid later to inputs, markets, and even the continuation of the IP as a platform (e.g. through registration). Besides, the results indicated that capacity building activities, especially those that can lead to generation of revenue –were of common interest to most actors in the IP. The results agree with observations by Nederlof et al. (2011) that IP members are motivated to participate when benefits are clear. The lowest attendance observed in Ouahigouya coincided with the peak of cropping season when demand for labor is high for weeding and other farm activities although similar trend was not observed in Koubri. These results suggest that timing of IP meetings will affect attendance and level of participation particularly when more participants are from a single actor category e.g. producers (farmers) in this case (Nederlof et al., 2011; Boogaard et al., 2013). There tended to be more participants at the IP meetings in Koubri than in Ouahigouya, which could be partly attributed to the proximity of the communities to the IP meeting venue. In terms of gender of the participants, men accounted for at least 60% of the total participants (Figure 2a and b) in both locations. The proportion of women at the IP meetings in Ouahigouya tended to be slightly higher than in Koubri. The number of women at the meetings declined after the IP meeting of March 2012 and then picked up as from March 2013. The domination of men at the IP meetings could be attributed to cultural factors (Amankwah et al., 2012) as well as pertinence of the issues addressed at the meetings to women These results suggest that the design and planning of participatory approaches such as IPs may need to be adapted to the local context to ensure inclusion, and not exclusion, of marginalized groups, that is, women in this case.

In terms of actors' group, the producers accounted for between 30 and 65% of the total participants at the IP meetings in both locations (Figure 3a and b). The actors' group consistently represented at the IP meetings were the producer (crop and livestock smallholder farmers), trader, processor, technical service, researcher and development practitioner (mainly NGOs). The credit agency was only present in one IP meeting (March 2013) in Koubri when the main issue discussed at the meeting was on commercialization and marketing of agricultural produce. The credit agency was consistently present at the IP meetings in Ouahigouya. The development practitioners (NGOs such as SNV and FNGN), traders, and researchers were consistently present at all the IP meetings (Figure 3a and b). The participation of the technical services, mainly from department of agriculture

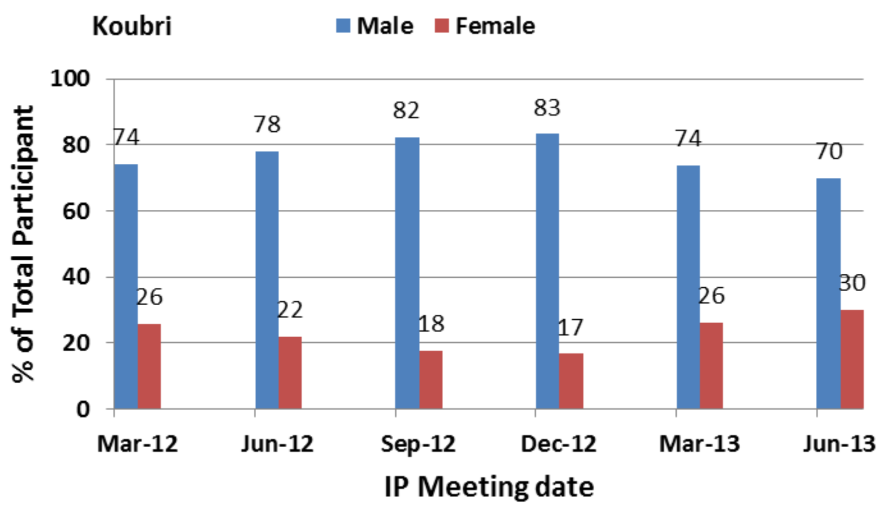

Figure 2a. Gender of the participants at the IP meetings in Koubri.

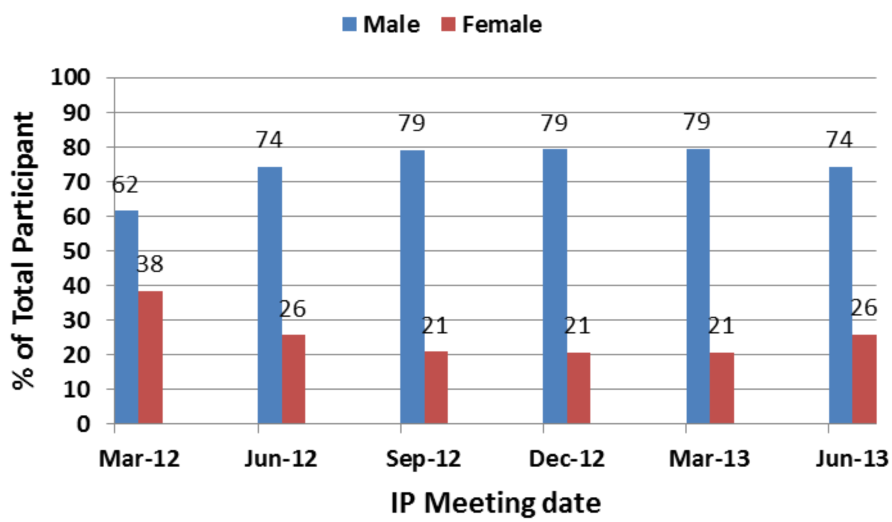

Figure 2b. Gender of the participants at the IP meetings in Ouahigouya.

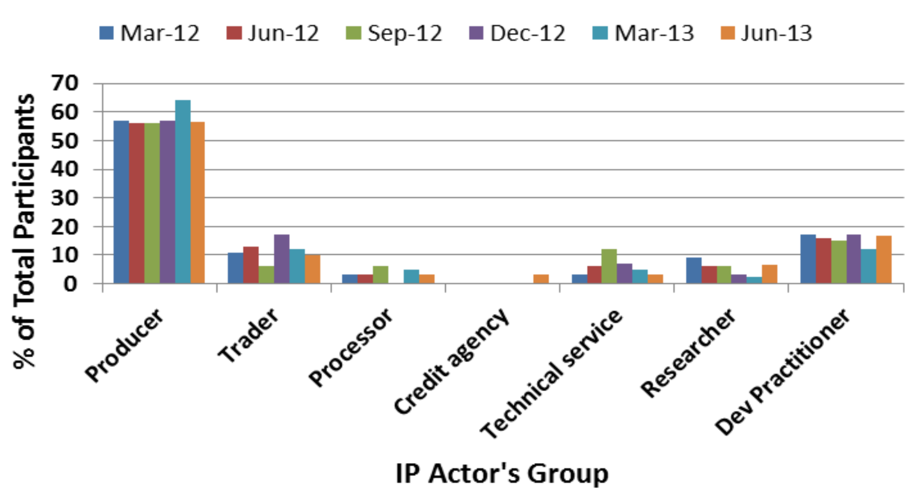

Figure 3a. Participants at the IP meetings in Koubri by actors' group.

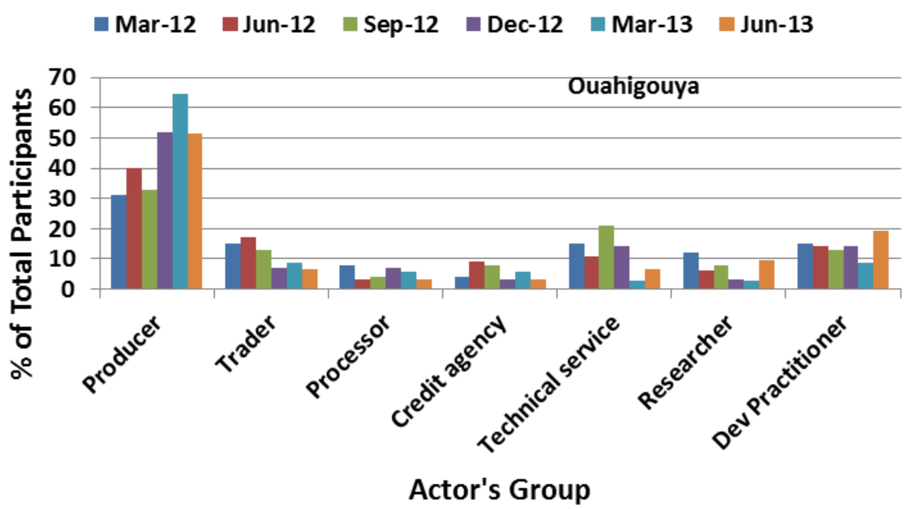

Figure 3b. Participants at the IP meetings in Ouahigouya by actors' group.

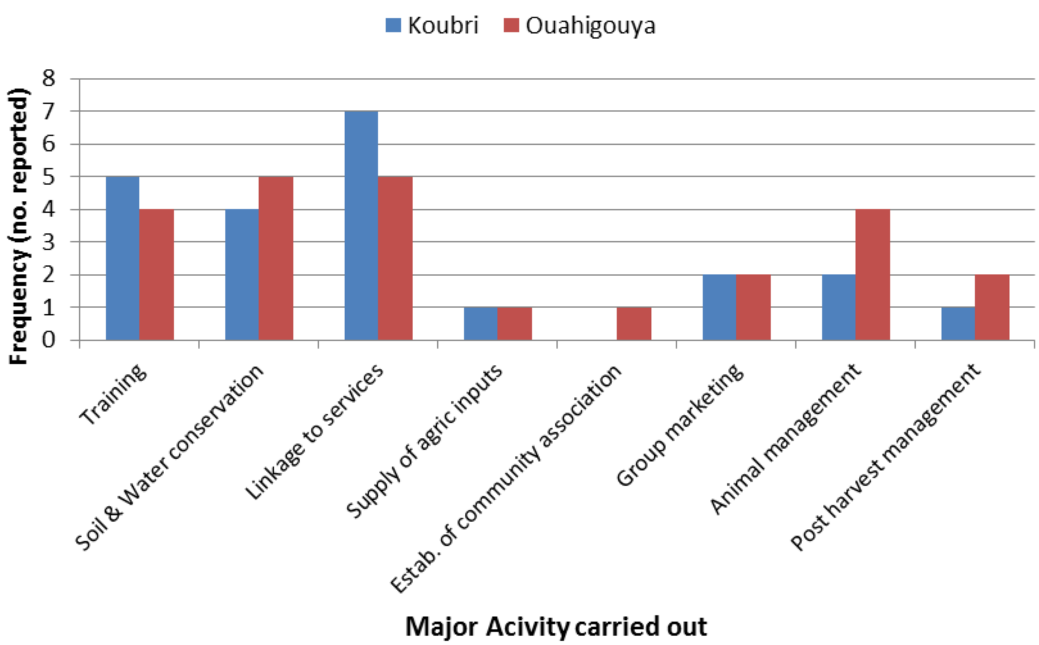

Figure 4. Major activities carried out by the innovation platform in Koubri and Ouahigouya as reported by the IP members in the activity protocol.

in the local district tended to fluctuate. In Ouahigouya, the presence of the technical service declined in the last two meetings (March and June 2013) probably due to dwindling interest. From experience, participation of this actor group is often based on direct benefits, for example payment of per diems or sitting allowances which should be discouraged as it is not sustainable for the IPs (Nederlof et al., 2011).

Major activities identified and carried out by the IP in Koubri and Ouahigouya included training, soil and water conservation initiatives, linkages to financial and technical services for technical information on agricultural production practices and access to credit, supply of agricultural inputs, group marketing (particularly of onion), animal management and post-harvest management (Figure 4). Activities such as group marketing of onions, animal management - particularly vaccination of cattle against Contagious Bovine Pleuro Pneumonia (CBPP) through contact with technical services, and capacity building, were some activities that the farmers' associations in the communities could not achieve before the establishment of the IP. The diversity in reported activities indicate that at the local level, IPs tend to focus on improving agricultural practices through joint experimentation and by linking farmers to markets and other stakeholders (Nederlof et al., 2011).

Training of the IP members was on commercialization and marketing of agricultural produce, soil and water conservation techniques (stone bunding, zai and composting). In addition, there was training on conservation and use of crop residues for profitable sheep fattening in Koubri. Soil and water conservation activities carried out by the IP members in both locations included stone bunding, digging well and composting. Though these activities are not new to the IP members, the innovative aspect was the collective implementation of these activities by members from different communities and the involvement of the technical agents providing necessary advisory services. This essentially entailed linking producers to technical services (agricultural, livestock and environmental services) for information on agricultural production practices such as improved soil fertility management techniques, crop pests control, diagnosis of animal diseases and to financial services (credit agency such as “Caisse Populaire”) for information on access to credit. The linkages were often facilitated by FNGN and SNV. In the interactions between the producers and credit agency, the conditions of access to loan were explained to the farmers even though the feedback from the producers was that the conditions are difficult to meet, particularly the need for collateral to obtain loan. Activities under animal management included construction of corralling pen and park for the animals to prevent damage to crops in the cropping season and to minimize theft. Post-harvest management of crop included storage of grains, collection of crop residues, particularly legume residue for animal feeding. Supply of agricultural inputs (fertilizer and improved seed of sorghum, millet and cowpea) was carried out only once by the national agricultural research institute (INERA) before the cropping season of 2012.

In both IP locations, linkage to technical and financial services had the highest frequency based on report of IP activity (Figure 4). This was followed by soil and water conservation, and then training on improved agricultural practices and commercialization. The activity on group marketing of onion was triggered by the sharp fall in price in Burkina Faso in 2012. These results confirmed that one of the objectives of the establishment of the IP is to promote better linkage of producers to technical and financial services (Hounkonnou et al., 2012; Boogaard et al., 2013). Whereas the technical advisory services in terms of crop and livestock production were of direct benefit to the farmers, the same was not the case regarding financial services where IP actors (mainly producers) were provided the information on conditions of access to credit but could not benefit from this service due to lack of collateral. Another lesson from the results is that building the capacity of the IP members is critical to sustaining their interest as this is of immediate benefits to the IP members. The sustained interest of the producers in the IP meetings could partly be explained by the training provided.

Members’ assessment of the IP functioning, activities and processes

The results of members’ assessment of IP functioning (Table 4) showed that location did not have a significant effect on key parameters, except for quality of facilitation in which case the average score for Ouahigouya was significantly lower than that of Koubri for the IP meeting of March 2012. This difference can be attributed to different FNGN teams responsible for the facilitation of the meeting at the two locations. This difference was corrected after the meeting of March 2012 by using the same team to facilitate the meetings. The results confirm the significant effect of the personnel involved in the facilitation on the IP functioning, and complies with the observation by Nederlof et al. (2011) that an innovation platform is as good as its facilitator. Effective facilitation of the IPs contributes to an enabling environment which can improve the quality of interactions between stakeholders. For all the parameters of IP functioning assessed, the lowest score (2.53±0.16 out of the maximum score of 5) was observed for the quality of facilitation in Ouahigouya for the meeting of March 2012 while the highest score (4.90±0.06) was for conflict resolution in the IP in Ouahigouya for the meeting in June 2013 (Table 4).

Overall, the members tended to score conflict resolution higher than other indicators. High scores for conflict resolution suggest that the IPs helped to keep a lid on previous conflicts that existed among members prior to the formation of IPs. The IP may have provided an avenue for conflicting factions to meet face to face and address issues which would not have been the case had the meetings not taken place. Better information sharing and understanding of key issues of common interest may contribute to the conflict prevention ability of the IPs. The results are in line with Cullen et al. (2014), who state that IPs tend to reduce tendencies for conflict among stakeholders. These results also confirm that conflict is minimal where stakeholders realize that they are dependent on each other for reaching a goal of common interest. Therefore, IP should always be established with clear goals and members should be composed of those with common interests otherwise IPs can become arenas for struggle when there are conflicting and competing interest. For example, Boogaard et al. (2013) observed that stakeholders with vested interests can be resistant to change thereby causing conflict.

The scores by the members for understanding and relevance of issues addressed at the IP meetings were also consistently high after those for conflict resolution

Table 4. Members' assessment of performance of the innovation platform in Koubri and Ouahigouya, Burkina Faso from March 2012 to June 2013.

IP location	IP meeting and number of respondent	Indicators of IP performance					
		Understanding of IP goals and issues	Participation in decision making	Information flow and sharing among actors	Conflict resolution	Quality of facilitation of IP	Benefit of IP
Koubri	Mar 2012; n=31[‡]	3.89±0.08[b]	3.69±0.09[b]	3.74±0.08[b]	3.74±0.15[b]	3.62±0.07[ab]	3.54±0.08[b]
	Jun 2012; n=30	4.68±0.07[a]	4.22±0.08[a]	4.67±0.08[a]	4.60±0.13[a]	3.78±0.10[ab]	4.18±0.11[a]
	Sep 2012; n=26	4.17±0.08[ab]	4.02±0.10[a]	4.06±0.12[ab]	3.96±0.13[b]	4.03±0.12[a]	4.0±0.12[a]
	Dec 2012; n=24	4.21±0.11[b]	4.21±0.13[a]	4.23±0.16[ab]	4.17±0.17[ab]	4.07±0.15[a]	4.10±0.13[a]
	Mar 2013; n=26	3.901±0.11[b]	3.62±0.09[b]	3.84±0.09[b]	4.81±0.08[a]	3.61±0.08[ab]	3.40±0.08[b]
	Jun 2013; n=18	4.58±0.19[a]	4.28±0.15[a]	4.47±0.15[a]	4.61±0.16[a]	4.19±0.16[a]	4.25±0.14[a]
Ouahigouya	Mar 2012; n=25	3.75±0.13[b]	3.31±0.09[b]	3.75±0.16[b]	3.64±0.20[b]	2.53±0.16[b]	3.21±0.12[b]
	Jun 2012; n=26	4.33±0.13[a]	3.98±0.11[a]	4.40±0.12[a]	4.31±0.22[a]	3.65±0.10[a]	4.06±0.08[a]
	Sep 2012; n=18	3.94±0.19[ab]	3.83±0.22[ab]	3.58±0.28[b]	3.78±0.13[b]	3.67±0.21[a]	3.81±0.21[a]
	Dec 2012; n=25	4.14±0.09[a]	3.99±0.34[a]	4.32±0.15[a]	4.08±0.18[ab]	3.66±0.12[a]	3.68±0.11[a]
	Mar 2013; n=17	4.12±0.09[a]	3.51±0.09[b]	3.64±0.09[b]	4.71±0.19[a]	3.32±0.08[b]	3.35±0.08[b]
	Jun 2013; n=22	4.16±0.10[a]	4.04±0.13[a]	4.18±0.09[a]	4.90±0.06[a]	3.98±0.13[a]	4.20±0.15[a]

The score is between 1 (lowest) and 5 (highest). Results presented are means ± standard error. [a,b]Values with different superscript letters denote significant difference (p < 0.05) between means within the column for each IP location. [‡]March 2012 meeting is the third IP meeting.

which affirms the relevance of IP approach in bringing multi-stakeholders together to achieve a common goal. Having a common understanding of the goals of the IP by the members is critical to its effective functioning because variations in understanding can lead to different interpretations of the platforms' goals and can lead to conflict (Boogaard et al., 2013; Swaans et al., 2014). For all the indicators, the mean scores tended to increase with the lifespan of the IP, that is the longer the lifespan the higher the score given by the members for its performance. These results are expected because with passage of time the relevance and benefit of IP activities become clear and concrete. These results also demonstrate that it may take reasonable length of time for IP to deliver concrete benefits, which implies that IP approach is not a "quick-win" approach. This raises the challenge of sustainability of IPs which are often established and funded by projects of short term duration.

The results of the regression analyses of the six parameters of IP functioning (response variables) on the independent variables (IP location (Koubri and Ouahigouya), period of IP meeting, actors' group and gender) are presented in the equations for the average scores below for each indicator (only variables that are significant at $p<0.05$ are included; means ± standard error). These regression results should be interpreted with caution as they were based on perception of the IP members and the R^2 is generally low for all the indicators. In the equations, the IP location, meeting dates, actors' group and gender are abbreviated as follows: L1 = IP location Koubri; L2 = IP location Ouahigouya; IP3 = Meeting in March 2012; IP4 = Meeting in June 2012; IP5 = Meeting in September 2012; IP6 = Meeting in December 2012; IP7 = Meeting in March 2013; IP8 = Meeting in June 2013; Actor2 = Producer; Actor3 = Trader; Actor4 = Processor; Actor 7 = Credit agency; Actor 8 = Technical services; Actor 10 = Researcher; Actor11 = Development practitioners; Male = Gender1; Female = Gender2.

1. Understanding and relevance of IP issues: 4.18±0.07 + 0.20±0.10 *IP8* - 0.36±0.13 *Actor8* (R^2 = 0.15)
2. Participation in decision making: 4.01±0.06 - 0.20±0.06 *L2* + 0.29±0.09 *IP6* + 0.36±0.10 *IP8* - 0.24±0.11 *Actor3* - 0.30±0.12 *Actor8* - 0.21±0.08 *Gender2* (R^2 = 0.43)
3. Information flow and sharing: 4.06±0.05 + 0.29±0.11 *IP6* + 0.29±0.11 *IP8* - 0.66±0.14 *Actor8* (R^2 = 0.41)
4. Conflict resolution: 4.33±0.05 + 0.51±0.14 *IP8* - 0.69±0.31 *Actor7* - 0.68±0.17 *Actor8* (R^2 = 0.35)
5. Facilitation of IP: 3.80±0.06 - 0.37±0.07 *L2* + 0.24±0.10 *IP6* + 0.51±0.11 *IP8* - 0.38±0.11 *Actor3* (R^2 = 0.46)
6. Perceived benefit: 3.86±0.05 - 0.17±0.07 *L2* + 0.48±0.10 *IP8* - 0.28±0.11 *Actor3* + 0.47±0.20 *Actor10* (R^2 = 0.41)

From the equations, gender only had significant effect in the regression equation for participation in decision making where women gave lower score than men. The results confirm again that the IP meetings were dominated by men in terms of participation in decision making which could be attributed to socio-cultural factors as well as the high proportion of male at the meetings. To ensure gender equity in participation and contribution in decision making in the IPs, it is necessary to involve more women

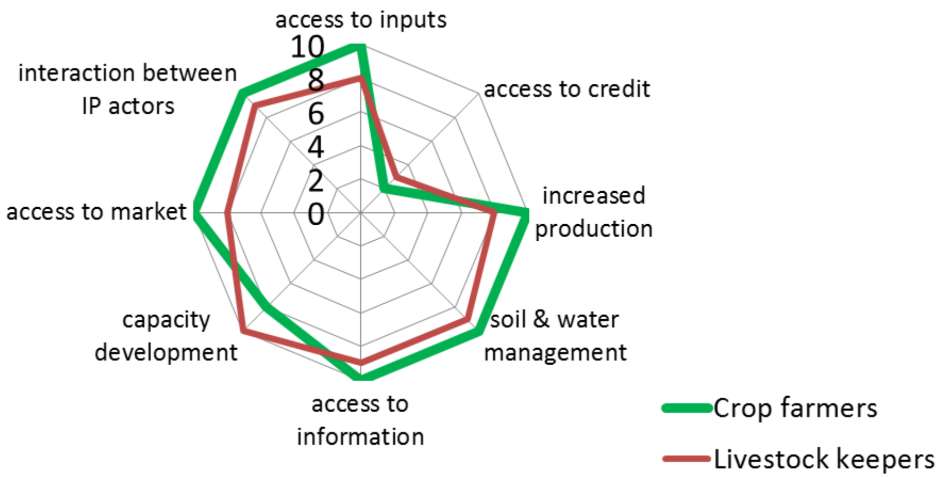

Figure 5a. Members' self-assessment of IP in Koubri.

than the present situation by addressing issues of interest to them (Klerkx et al., 2010; Nederlof et al., 2011; Adekunle et al., 2012). Compared to IP in Koubri, the members of IP in Ouahigouya gave lower score to participation in decision making, quality of facilitation of IPs and perceived benefit of the IP. This may be attributed to long exposure to development projects by people in Ouahigouya compared to Koubri which might have made the IP members in this site to be more critical in their assessment of the IP activities. In both IP locations, the technical services group gave lower score to the six indicators compared to the producer's group. This shows that the technical services are more critical of the performance of the IP than the producers. This was expected as most of the IP activities were targeted at the crop and livestock producers. To engage the technical services in the IPs and sustain their interest, it is necessary to include activities that are of interest to them for example training in their technical domains or remunerate them for services provided to producers in the IP. The results also showed that the researchers gave higher score for the perceived benefit of the IPs than the producers. Since this group and the development practitioners were responsible for the establishment of the IPs, they might have strong justification to show that the IP is beneficial to all the actors. The scores for the six indicators for the IP meeting in June 2013 were significantly higher than the scores for the meeting in March 2012 which again confirms that IP tends to perform better with more time to carry out its activities. However, it needs to be stated that the scores seem to fluctuate and the increase over time was not linear.

Achievements of the innovation platforms according to the producers

To further assess the perceived benefits of IP by the producer groups (crop farmers and livestock keepers), we presented their scores of different achievements of IP in the two study sites in Figure 5a and b. According to the crop farmers, the highly rated (with average score of between 8 to 10) perceived benefits of IP in Koubri were access to inputs, access to market, improved soil and water management, better interaction between IP actors, access to information and increased production (crop yields) while the livestock keepers rated highly capacity development, access to inputs, better interaction between IP actors, access to information and increased production as the perceived benefits (Figure 5a). Generally, the crop farmers and livestock keepers in both sites scored high on most issues, except for access to credit (average scores of 2 and 3 for crop farmers and livestock keepers, respectively) due to the persistent problem of accessing credit from the micro-finance NGOs at the study sites despite the repeated attempts of the IP to engage with the NGOs. Generally, the crop farmers tended to rate a bit more highly the perceived benefits of the IP in Koubri than the livestock keepers though the overall trend for both is similar. This trend could be partly attributed to low representation of the livestock keepers (agro-pastoralists) as they are recent immigrants in Koubri. Besides, the IP meeting agenda in Koubri tilted heavily in favor of the crop farmers who dominated the meetings. The results for the IP in Ouahigouya (Figure 5b) were the opposite of those of Koubri as the livestock keepers rated more highly the perceived benefits of IP in Ouahigouya than the crop farmers. The livestock keepers scored access to inputs, interaction between actors, capacity development, and access to information very highly (average scores of ten out of ten) whereas crop farmers only scored access to information very highly. Again, access to credit had the lowest score of about five out ten for both crop farmers and livestock keepers in Ouahigouya as it was in Koubri. The livestock keepers in Ouahigouya also rated quite low access to market based on their experience of low prices

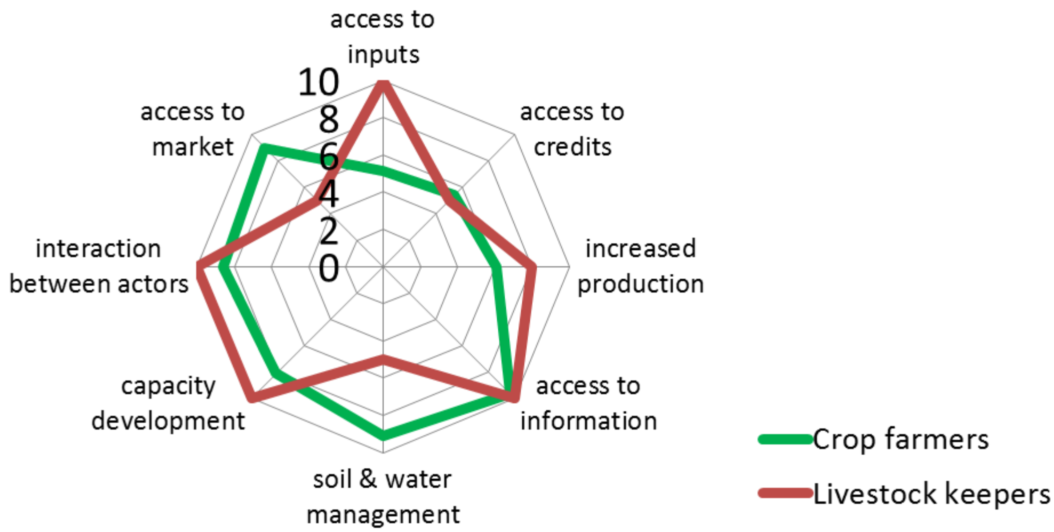

Figure 5b. Members' self-assessment of IP in Ouahigouya.

for their animals. According to them, the intermediaries who come to the villages to buy live animals often offered low prices and were the ones making most profit. To address this problem, training was organized on group marketing to strengthen their negotiating power but this faced the problems of trust among the livestock keepers and the inability to wait for buyers with good prices as they are often compelled to sell their animals to meet urgent needs. Generally, these results suggest that IP is perceived as successful by the producers except for access to credit and that it is able to address technical, organizational and institutional constraints. Besides, the results support the general view that benefits of platform participation should be visible as incentive for active engagement of the members. Adekunle et al. (2012) observed that platforms centred on value chain development have inherent financial benefits which may motivate participation by the actors but the incentives for participation is less visible in platforms on natural resource management as concrete benefits tend to be on a long-term.

Implications of findings from the study for IP approach

From the results of this study, the key emerging issues regarding IP approach for operationalization of Integrated Agricultural Research for Development are:

1. For agricultural research and development, it is important that stakeholders themselves perceive the IP as beneficial. Our results suggest that this seems to be the case even though the short term benefits such as capacity building to increase the skills and knowledge of the IP members seem more obvious than long-term outcomes such as improved crop and livestock productivity.
2. Key factors identified through this study for the performance of IPs were quality of facilitation, location, the period (season) of IP meeting and gender. These need to be taken into account when designing and establishing IPs. Facilitation is critical to the IP process and functioning as has been argued by several others (Klerkx et al., 2010; Adekunle et al., 2012; Schut et al., 2015).
3. IPs need time to mature and to become fully functional in order to deliver concrete outcomes, which implies that the IP process needs to go along with short terms benefits such as a training/capacity building.
4. IPs tends to focus initially on production as shown by our results on activities carried out by the IP members but this tends to change over time whereby the platforms address more institutional issues. To achieve this, it is important that the facilitators keep the process focused on issues of shared/common interest. For example, this could be done by establishing farmer producer groups alongside IPs so that IP can focus more on institutional issues instead of issues mainly related to production.
5. There is a risk that IPs will reinforce the current Agricultural Research for Development regime (Schut et al., 2015). However, there are indications in the study that institutional changes did occur, which will need continuous attention and willingness from stakeholders from the start not only to support and help others, but also to challenge and be critical of their own practices.
6. This study relies heavily on a self-assessment of participants. There may be a risk that this may not necessarily comply with what happened 'in reality'. However, we would argue that it is in the first place the stakeholders themselves who need to see the added value of the IP and be willing to invest in the process.

Therefore, their perceptions provide an important element in assessing IPs.

7. Simple participatory monitoring and evaluation can provide useful information to steer and adapt/correct the IP process if necessary. The tools we used in this study are very simple and could be applied and documented by the facilitation staff themselves even though they often do not see M&E as being their task, which poses a challenge to the systematic documentation of IP processes and performance (Swaans et al., 2013).

Conclusions

Assessment of the functioning of two IPs in Koubri and Ouahigouya, Burkina Faso shows that innovation systems approach is relevant and important for effective linkages between different actors for better access to technical and financial services, and for building capacity of the members. The performance of the IPs seems to improve with the lifespan which underscores the necessity of long-term plan for the establishment of IPs. To ensure effective participation of different actors at the IP, issues being addressed should be of common interest and should be clearly articulated. Therefore, there should be concerted efforts by the facilitators of the IP to engage all the actors and avoid domination by any group. Facilitation is critical to IP performance as shown by the results of the members' assessment. In addition, a systematic monitoring of IP functioning is indispensable for assessment of its performance and output, and this should be accorded the right place in the running of the IPs.

Conflict of Interests

The authors declare that they do not have conflict of interests

ACKNOWLEDGEMENTS

This research was carried out through the CGIAR Challenge Program on Water and Food (CPWF) in the Volta Basin with funding from the European Commission (EC) and technical support from the International Fund for Agricultural Development (IFAD).

REFERENCES

Adekunle AA, Ellis-Jones J, Ajibefun I, Nyikal RA, Bangali S, Fatunbi O, Ange, A (2012). Agricultural Innovation in Sub-Saharan Africa: Experiences from Multiple-Stakeholder Approaches. Forum for Agricultural Research in Africa (FARA), Accra, Ghana.

Amankwah K, Klerkx L, Oosting SJ, Sakyi-Dawson O, van der Zijpp AJ, Millar D (2012). Diagnosing constraints to market participation of small ruminant producers in northern Ghana: An innovation systems analysis. NJAS - Wageningen J. Life Sci. 60-63:37-47.

Boogaard B, Schut M, Klerkx L, Leeuwis C, Duncan A, Cullen B (2013). Critical issues for reflection when designing and implementing Research for Development in Innovation Platforms. Report for the CGIAR Research Program on Integrated Systems for the Humid Tropics. Knowledge, Technology & Innovation Group (KTI), Wageningen University & Research centre, the Netherlands.

Cadilhon J (2013). A conceptual framework to evaluate the impact of innovation platforms on agrifood value chains development. Paper presented at the 138th European Association of Agricultural Economists Seminar on Pro-poor Innovations in Food Supply Chains, Ghent Belgium September 11-3, 2013.

Cullen B, Tucker J, Snyder K, Lema Z, Duncan A (2014). An analysis of power dynamics within innovation platforms for natural resource management. Innov. Dev. 4(2):259-275.

Douxchamps S, Ayantunde A, Barron J (2014). Taking stock of forty years of agricultural water management interventions in smallholder systems of Burkina Faso. Water Res. Rural Dev. 3:1-13

Engel PGH (1995). Facilitating Innovation: an Action-oriented Approach and Participatory Methodology to Improve Innovative Social Practice in Agriculture. Wageningen Univer. Wageningen, The Netherlands.

Hounkonnou D, Kossou D, Kuyper TW, Leeuwis C, Nederlof ES, Röling N, Sakyi-Dawson O, Traoré M, van Huis A (2012). An innovation systems approach to institutional change: Smallholder development in West Africa. Agric. Syst. 108:74-83.

Kilelu CW, Klerkx L, Leeuwis C (2013). Unravelling the role of innovation platforms in supporting co-evolution of innovation: Contributions and tensions in a smallholder dairy development programme. Agric. Syst. 118:65-77.

Klerkx L, Aarts N, Leeuwis C (2010). Adaptive management in agricultural innovation systems: The interactions between innovation networks and their environment. Agric. Syst. 103:390-400.

Klerkx L, van Mielo B, Leeuwis C (2012). Evolution of systems approaches to agricultural innovation: Concepts, analysis and interventions. In: Farming systems research into the 21st century. The new dynamic, ed. I. Darnhofer, D. Gibbon, and B. Dedieu, Dordrecht, The Netherlands: Springer. pp. 457-483.

Leeuwis C, van den Ban A (2004). Communication for Rural Innovation: Rethinking Agricultural Extension. Oxford: Blackwell Science.

Lundvall BÅ (2011). Notes on Innovation Systems and Economic Development. Innov. Dev. 1:25-38.

Nederlof S, Wongtschowski M, Van der Lee F (eds.) (2011). Putting heads together: agricultural innovation platforms in practice. KIT publishers: Amsterdam, the Netherlands. Bulletin P. 396.

Pali P, Swaans K (2013). Guidelines for innovation platforms: Facilitation, monitoring and evaluation. ILRI Manual 8. Nairobi, Kenya: ILRI.

Pretty J, Toulmin C, Williams S (2011). Sustainable intensification in African agriculture. Int. J Agric. Sustain. 9(1):5-24.

van Rooyen A, Homann-Kee Tui S (2009). Promoting Goat Market and Technology Development in Semiarid Zimbabwe for Food Security and Income Growth. Trop. Subtrop. Agroecosystems 11(1):1–5.

Schut M, Klerkxl L, Sartas M, Lamers D, Campbell MMC, Ogbonna I, Kaushik P, Atta-Krah K, Leeuwis C (2015). Innovation platforms: Experiences with their institutional embedding in agricultural research for development. Exp. Agric. 25 p.

Statistical Analysis System Institute (1987). SAS/STAT for Personal Computers, SAS Institute, Inc., Cary, NC.

Swaans K, Cullen B, van Rooyen A, Adekunle A, Ngwenya H, Lemma Z, Nederlof S (2013). Dealing with critical challenges in African innovation platforms: lessons for facilitation. Knowl. Manage. Dev. J. 9:116-135.

Swaans K, Boogaard B, Bendapudi R, Taye H, Hendrickx S, Klerkx L (2014). Operationalizing inclusive innovation: lessons from innovation platforms in livestock value chains in India and Mozambique. Innov. Dev. 4(2):239-257.

academicJournals

Vol. 11(33), pp. 3154-3161, 18 August, 2016
DOI: 10.5897/AJAR2015.10678
Article Number: 2A882F760054
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Anthocyanin content, flavonoid, color and physicochemical characteristics of dried jabuticaba residue

Claudia de Andrade Moura[1,2]*, Daiane Luckmann[3], Jessica Cristina Urbanski Laureth[3], Divair Christ2 , Gilberto Costa Braga[3], Dalva Paulus[1], Silvia Renata Machado Coelho[2] and Lucas Felipe Francisco[1]

[1]Federal Technological University of Paraná, Dois Vizinhos, Paraná, Brazil.
[2]Western Paraná State University - UNIOESTE/CASCAVEL/CCET/PGEAGRI, Paraná, Brazil.
[3]Western Paraná State University - UNIOESTE, Marechal Cândido Rondon, Paraná, Brazil.

Received 23 November, 2015; Accepted 19 April, 2016

The search for alternatives to exploit nutritional and functional properties of agro-industrial by-products has stimulated the development of researches. Agro-industries have targeted different native fruits, such as jabuticaba, to produce juices or candies. Jabuticaba have high nutritional value, and its industrial residues can also contain nutritional compounds that may be recovered. The objective of this work was to verify storage effects on anthocyanin and flavonoid contents, color and physical-chemical features from agro-industrial by-products of two genotypes of jabuticaba (Clevelândia and Verê) and two industrial processes of juice extraction (crushing and steam). By-product samples were ground and the powder was packaged on vacuum, and was then stored during 135 days. The powdered peel of both Jabuticaba genotypes are rich in flavonoids and steam extraction was more effective to obtain the peel. Clevelândia genotype had higher anthocyanin content, but both genotypes showed high levels of this compound. Color quality is enhanced when dehydrated with attractive pigmentation for blends in food. Thus, jabuticaba peel has significant nutritional and functional levels, being a good source of fiber, ash, natural pigment and phenolic compounds. It can be used in food products such as bioactive ingredient.

Key words: *Myrciaria cauliflora*, shelf life, by-products.

INTRODUCTION

Jabuticaba (*Myrciaria cauliflora* Berg.) is a Brazilian native fruit, which spontaneously occurs all over the country, but its chemical contents are barely unknown (Lima, 2008; Sasso et al., 2010). It is a subtropical fruit, which contains high nutritional value, but its destination is still concentrated in *in natura* consumption (Marquetti,

* Corresponding author. Email: claudiaamk@yahoo.com.br

2004). Currently, there has been an increasing agro-industrial interest in this fruit due to its antioxidant activity, which is higher than grape's one, emphasizing that the fruit peel concentrates its higher bioactivity.

Agro-industrial activity generates huge amounts of residual biomass, which is a potential source of antioxidant compounds, including phenolic compounds (Schieber et al., 2001). Using agro-industrial residues as antioxidant source reduces destination costs and adds value to final product, representing an economically viable alternative. Phenolic compounds are secondary metabolites derived from fruit phenylpropanoid metabolic pathways (Randhir et al., 2004). As natural antioxidants, phenolic compounds may play important functional role in human organism, reducing oxidative stress at cell level (Balasundram et al., 2006; Othman et al., 2007; Babbar et al., 2011). Agro industrial residues consist of raw material remaining parts, such as peels and seeds. Researches have shown that peel is usually the part of fruit that concentrates the highest level of phenolic compounds (Marks et al., 2007; Ribeiro et al., 2008; Mikulic-Petkovsek et al., 2010).

Dehydration preserves vegetables and plays an important role in its final quality, but if done improperly, it may cause food decay and considerably change its physicochemical properties (Faroni et al., 2006). Jabuticaba is a dark colored fruit, rich in anthocyanin soluble pigments, which, through dehydrating, add color and nutritional value to other foods, which quality essentially relies on nutritional facts, and color is a relevant final aspect (Alves and Silveira, 2002). However, the stability of dehydrated products can be changed during storage, which alters their physico-chemical properties. Although anthocyanins are widely distributed in nature, their commercial use is restricted. Probably it is explained by its sensitivity to heat, which accelerates decay, loss of color (caused by the presence of ascorbic acid) and sugars (Bobbio, 2001). The influence anthocyanin degradation, according to Seravalli (2004), occurs due to several factors, among them are: pH, temperature, enzymes, ascorbic acid, oxygen, sulfur dioxide and metal ions (especially iron). These can occur during harvesting, the plant extraction, processing and storage of food. Preventive measures are necessary at all stages of obtaining such compound.

Due to the facts described above and to jabuticaba nutritional importance, the objective of this work was to evaluate storage effect on anthocyanin and flavonoid contents, color quality and physico-chemical features of juice extraction residues of two jabuticaba genotypes (Clevelândia and Verê) processes of crushing and steam.

MATERIALS AND METHODS

Samples

This study made use of Jabuticaba (*Plinia cauliflora*) fruits identified as Cl genotype, from a farm in Clevelândia, Paraná, and VR genotype from a farm in Verê, in the same state. Manual parcel harvest occurred in the morning, and the fruits were immediately wrapped in 36 x 55,5 x 31 cm high density polyethylene to be transported to UTFPR-DV agro industry where they were processed on the same day. In the process, jabuticabas were classified, and those fruits without physical damage or decay were selected. Afterwards, they were washed in tap water and sanitized with 100-ppm sodium hypochlorite solution. Then, the fruits were washed in distilled water and drained for 10 min.

Jabuticaba process and residue obtaining

There were tests to evaluate jabuticaba juice residues resulting from both crushing and forced steam extraction methods. The flowchart (Figure 1) demonstrates jabuticaba extraction process and residue dehydrating (stage 1), and powder residue storage. The equipment used to extract jabuticaba juice through steam had 40 kg h^{-1} maximum capacity, and extraction temperature was 70°C. Both genotype residues are composed of peels and about 3% seeds. Obtained peels were drained, eliminating juice that remains after steam extraction, then they were stored in a -18°C freezer, until analyses and its dehydration. Crushing process happened in a stainless steel fruit de-pulping machine with polished aluminum nozzles whose capacity was up to 100 kg h^{-1}. The same hygiene procedures were performed, separated and drained peels right after storage in a -18°C, until the analyses and dehydration.

Residue drying and storage

The obtained residues were dehydrated in a forced-air dryer, heated by liquefied petroleum gas (LPG). Drying temperature was 70°C until samples reached 10% base humidity. Author's preliminary studies determined this drying temperature. In this work, jabuticaba sample showed no significant change on total phenolic content or physico-chemical features until this drying temperature.

A semi-industrial blender (Skymsen, modelo LV -1,5) grinded the dehydrated peels for five minutes, then 60-mesh sifts sifted powder, separating bigger particles. Vacuum package (PP plastic transparent) wrapped all the powder and researchers placed packages on stainless steel shelves under room temperature without incidence of direct light. The analyses were performed at initial time, then at 45 and at 135 days.

Physicochemical analyses

Total titratable acidity

Acidity was measured by potentiometric method, Adolfo Lutz Institute (2008) is indicated in the case of dark or strongly colorful solutions. 2.5 g of samples were weighed in a 100 mL Becker and diluted it 50 mL ultrapure water. Then, the electrode was immersed in the solution and began titrating the sample with NaOH 0.1 M until 8.2- 8.4 pH level. 100 g^{-1} citric acid was expressed result, using citric acid equivalent = 64, according to Lima et al. (2008).

pH

pH was determined and previously calibrated with 4.0 and 7.0 pH buffer solution pH meter (IAL 2008).

Total soluble solids

In the determination of total soluble solids (SST), the verification

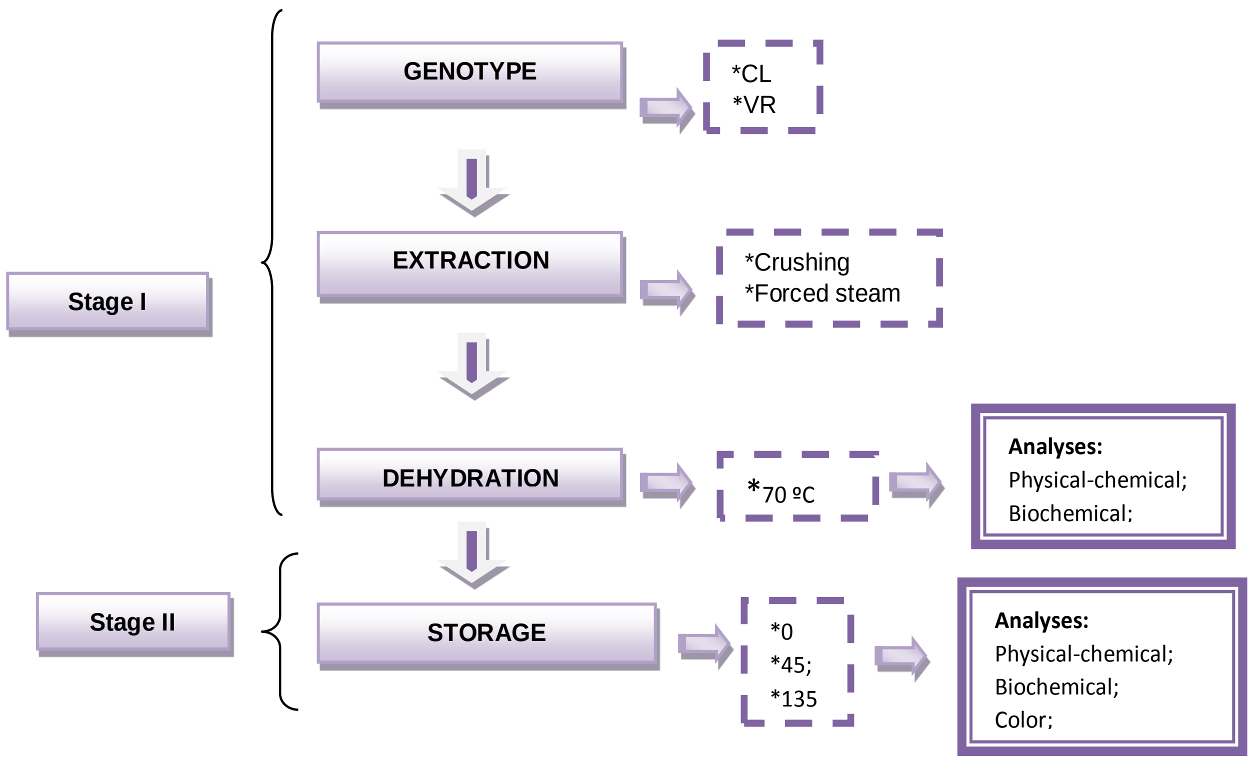

Figure 1. Jabuticaba processing flowchart, dehydration and residue storage.

was according to Instituto Adolfo Luz methodology, the results are expressed in °Brix by digital refractometer. Samples were performed in triplicate.

Ash

Ash was determined by gravimetric method, according to Instituto Adolfo Lutz's physicochemical methods to analyze foods (2008). 5.0 g sample were weighed in a previously dried crucible of known mass. Firstly, the samples were heated to 550°C kiln to incinerate during four hours, then cooled under room temperature in desiccators and once again weighed. The results were expressed in g 100 g^{-1}.

Humidity content

Humidity content was determined by gravimetric method in a 105°C oven until constant mass of dry mass, according to Adolfo Lutz Institute (2008). 2.0 g samples were weighed in a previously weighed porcelain vessel. After that, samples went to an oven with air circulation until constant weight (six hours average). The results were expressed in g $100g^{-1}$.

Flavonoids and total anthocyanins

Extract preparation

The extracts were obtained by cold hydro alcoholic extraction method, according to Vedana (2008). 1 g sample was immersed in 10 mL 80% ethanol weighed, placed in a 15 mL falcon, then placed in an ultrasound for 20 min. Afterwards, each sample was taken to 3500 rpm centrifuging for 20 min. Supernatants were transferred to another falcon and kept under -18°C temperature until the analyses was performed ten days later.

Total flavonoids

They were determined according to Chang et al. (2002), with some changes. In 0.5 mL extract, there was the addition of 4.3 mL 80% ethanol in water (v/v), 0.1 mL $AlCl_3$ (10% w/v) and 0.1 mL potassium acetate (20% w/v). A parallel control series was performed with 80% ethanol replacing $AlCl_3$. After 40 min in darkness under room temperature, absorbance was measured at 415 nm. The results were expressed in mg g^{-1} of fresh weight, equivalent quercetin (EQ), adjustment of calibration curve for quercetin was calculated.

Total anthocyanins

Anthocyanins were determined through differential pH methodology proposed by Lee et al. (2005), which consisted firstly of preparing pH 1 (KCl 0.025 M) and 4.5 pH ($C_2H_3NaO_2$ 0.4 M) buffer solutions. After preliminary dilution tests, 1 ml extract and 19 mL of respective buffers were added. 20 min later, the absorbance levels of both were measured at 510 and 700 nm. The white one was prepared with ultrapure water. The level of total anthocyanins (AT, mg Ci-3-Gly L^{-1}) was obtained through equation 1 level and expressed in 100 g jabuticaba peels:

$$TA = (A \times MW \times DF \times 10^3) \div (\epsilon \times I) \quad (1)$$

Where, A = (A_{510nm}- A_{700nm}) pH 1 – (A_{510nm}- A_{700nm}) pH 4.5; MW = 449.2 g mol^{-1} for cianidine-3-glicoside; DF = dilution factor; I = light path in cm; ε = 26.900 molar extinction coefficient (L x mol^{-1} x cm^{-1}) ; 10^3 = g to mg conversion factor.

Color

Color was determined by direct reading of L*, a* and b* coordinate reflectance, applying CIELAB scale in a Konica Minolta CR-410 tristimulus colorimeter to 10º/D_{65} in triplicate measurements. Color angle or tone (H*), calculated by Equation 2, is the most familiar color aspect that can be described and identifies colors as red, green, blue or yellow. It starts on +a*axis and expressed in degrees: 0° for red (+a*), 90° for yellow (+b*), 180° for green (-a*) and 270° for blue (-b*). Chroma index (C*), calculated by Equation 3, indicates tone intensity or purity, independently on how light or dark the color is. The higher its tone, the more intense the color is, or highly chromatic seeming luminous or concentrated, the levels.

$$H^* = tan^{-1}\left(\frac{b^*}{a^*}\right) \quad (2)$$

$$C^* = \sqrt{[(a^*)^2 + (b^*)^2]} \quad (3)$$

Sample color location and even statistics are not enough to express if color differences are visually distinguishable. These color differences (ΔE^*_{ab}) are important to evaluate numerical and visual relations (CIE, 1995) and may be calculated by the distance between the two spots in tridimensional room defined as colorimetric parameter a*, b*, c* and L* mathematically described by Equation 4.

$$\Delta E^*_{ab} = \sqrt{(\Delta L^*)^2} + (\Delta a^*)^2 + (\Delta b^*)^2 \quad (4)$$

Statistical analysis

The experiment was conducted according to a wholly randomized outlining. A variance analysis was applied and a Tukey test was applied on the parameters, and the results were significant for F test. Every analysis used p<0.05 significance level and SISVAR statistical package.

RESULTS AND DISCUSSION

Acidity, pH, soluble solid content (SST), ash and humidity results of Jabuticaba peels (both genotypes) subjected to steam and crushing treatments followed by dehydration are shown in Table 1.

It was observed that independent of the way fruit is processed, Verê jabuticaba residue showed higher ATT than Clevelândia sample residue. At the end of storage (135 days), residues from both genotypes demonstrated an increase of titratable acidity, but this occurred just for those resulting from steam extraction. Most likely, this increase occurred due to microbial activity, because higher humidity levels may have led to its development. pH result between Verê and Clevelândia genotypes in steam process and crushing process differed statistically, indicating that Verê genotype has less acid peel showing a more elevated acidity than Clevelandia genotype. Regarding storage time, it presented itself stable at initial time (0) until 135 days (from 2.99 upto 3,50 average between genotypes) (Table 1). It is emphasized that Vere genotype residue is more acid. However, it suffered changes in total titratable acidity, when the peel was separated by crushing at the time of 45 days and peel obtainment through steam separation at the time of 135 days. There is a notion that this may relate to humidity level in the value results above 15%, throughout times in study.

There were lower levels for jabuticaba peel (0,71 g citric acid 100 g^{-1}) in a work developed by Silva et al. (2013). However, contents verified by Marquetti (2014) (3.77 g citric acid 100 g^{-1}) in the peel flour and by Boari Lima et al. (2008) in jabuticaba Paulista and Sabará fresh pulp (1.37 and 1.67 g citric acid 100 g^{-1}) are similar to the obtained in this study for dehydrated residue.

Maximum acidity in flours, according to Brazilian laws, is 2.0 mL NaOH N 100 g^{-1}, but most samples of this experiment are not in these parameters because it varied from 2.02 to 4.91 mL NaOH N 100 g^{-1}. On the other hand, according to Soares et al. (1992), most bacteria, moulds and yeast grow in pH higher than 4.5.

SST showed higher contents in Clevelândia genotype peel (Table 1). For crushing process, no significant differences were observed between genotypes. Furthermore, steam process showed higher SST values if compared with crushing, indicating that crushing assisted in greater losses of solids. Nevertheless, SST may imply lower post-harvest potential of conservation, due to higher fermentation (Barros et al., 1996). For powdered peel humidity content, at genotype zero time both showed contents below recommendation by Brazilian law, which is below 15%.

Marquetti (2014) observed 8.63 humidity content in the preparation of cookie flour, close to residual contents seen in this study, corresponding to Verê genotype peel powder (9.80%) and Clevelândia (10.60%), at the beginning of storage. Also, the results is similar to that of Boekel et al. (2011), where rice and soy flours showed 8.60 and 10.3% humidity. Although, after 45 days of storage, humidity level increased (from 17.79 to 15.89%) to both genotypes and separation methods. This time variation was gradual and stable. However, samples stored for 135 days obtained stability closer to the control in its humidity, that is, the product (jabuticaba peel powder) from Clevelândia by steam method (14.41%) as well as powder obtained by crushing both genotypes (Verê 14.26% and Clevelândia 14.51%). There was a slight increase in humidity content, when compared with initial time, getting close to limit value of flours, because humidity contents above 14% may form lumps (Fernandes et al. 2008). Thus, after 135 days, powder residue obtainment kept its humidity content within Brazilian parameters (Brasil, 1978), which range from 5 to 15% humidity, making this product viable to be used in food mixes.

Ash content of the peel differed statistically (p<0.05),

Table 1. pH averages, total titratable acidity (ATT), soluble solids (SST), *in natura* peel humidity, jabuticaba peel ash stored for 135 days.

Physical-chemical	Storage (days)	Process residues			
		Steam		Crushing	
		Verê	Clevelândia	Verê	Clevelândia
Total titratable acidity (% citric acid)	0	3.53[a]	1.81[b]	3.74[a]	2.02[b]
	45	3.64[a]	1.80[b]	4.21[a]	2.32[b]
	135	4.91[a]	3.60[b]	3.65[a]	2.65[b]
Soluble solids (°Brix)	0	2.70[b]	3.21[a]	2.75[a]	2.56[a]
	45	3.03[b]	3.51[a]	2.61[a]	2.59[a]
	135	2.93[b]	4.26[a]	2.61[a]	2.63[a]
pH	0	3.06[b]	3.50[a]	3.10[b]	3.40[a]
	45	3.04[b]	3.50[a]	3.15[b]	3.35[a]
	135	2.99[b]	3.43[a]	3.15[b]	3.34[a]
Ashes (%)	0	5.75[a]	2.62[b]	4.96[a]	3.45[b]
	45	5.98[a]	3.51[b]	5.29[a]	3.73[b]
	135	2.64[a]	2.25[a]	3.39[a]	1.89[b]
Humidity content (% humid base)	0	11.89[a]	12.20[a]	9.80[a]	10.60[a]
	45	17.79[a]	17.93[a]	15.89[a]	15.98[a]
	135	18.43[a]	14.41[b]	14.26[a]	14.51[a]

between genotypes and Verê (Table 1), presenting higher values. After 135 days, both genotypes suffered losses and forms to obtain residues (steam and crushing), expected factor, but the expectation was that it remained stable. Perhaps there was interference that caused the oxidation of sulfates, carbonates, phosphates and silicates (mineral residues) that still remained in the samples up to that period. Regarding Verê genotype, it is noticeable that from 0 to 45 storage days, both crushing and forced steam resulting peel showed better results in comparison with Clevelândia genotype. This genotype had inverse response regarding peel obtainment form, since it is more favorable to crushing, after being stable for the first 45 days.

After being dried, the samples accompanied by their respective obtained residues from both extraction methods suffered losses during storage, but in spite of losses, it contains a higher residual ash content than that found by Silva et al. (2013) (0.51 g 100g^{-1}). However, Lima et al. (2008) expressed higher values which were close to that found in this present work for jabuticaba Sabará (4.40 g 100 g^{-1} dry mass) and Paulista (2.88 g 100g^{-1}) peels. Lenquiste et al. (2012) observed 3.52 g 100 g^{-1} in freeze-dried peels and the process applied in this work is efficient. Besides, ash values indicate quantity of mineral residue present in the peel (Marquetti, 2014). This process is also an alternative source of this element.

Jabuticaba peel demonstrated that it contains higher levels of flavonoids, differing statistically between cultivars when extracted by steam and crushing methods, and flavonoid contents reduced gradually from 0 to 135 days, showing oscillation on the 45th day in Clevelândia genotype by steam method, which is attributed to humidity. The degradation of these compounds is due to the conditions of high temperatures and humidity, as well as light, pH and others. All necessary measures have been taken so that these interfering conditions did not cause any damage to analysis. However, with the advent of drying, there is a sign that these compounds are concentrated (Figure 1). The highest flavonoid contents were in Clevelândia genotype when extracted by steam method with 16.81 mg g^{-1}, during 45 days of storage.

The differences between genotypes can be justified by the greater exposure of these peels to environmental factors, leading to greater incentives for the production of these secondary metabolites related to protection against abiotic stress, making them present higher or lower concentrations of flavonoids (Araújo, 2011). In a work performed by Marquetti (2014) with jabuticaba peel, the author emphasized that even after drying and dehydration, a great deal of these compounds remained and could be transferred to foods where they may work as bioactive ingredient. Abe et al. (2012), while evaluating various fruits as potential sources of bioactive compounds, observed lower contents (33.0 mg CE 100g^{-}

Table 2. Parameter average using CIELAB scale, to analyze jabuticaba color.

Treatments	TA	L*	C*	H*	ΔEab Color
In natura VR		23.46[a]	10.18[a]	7.04[b]	25.47[a]
In natura CL		20.25[b]	4.89[b]	171.13[a]	20.98[b]
GVRE	0	28.40±1.17[a]	12.53±3.39[a]	11.47±0.51[a]	31.11±2.43[a]
	45	28.62±0.98[a]	11.48±0.99[a]	12.01±0.57[a]	31.13±1.21[a]
	135	28.32±1.09[a]	13.57±2.71[a]	14.45±0.85[a]	32.91±2.06[a]
GCLE	0	26.88±1.15[b]	12.32±4.61[a]	10.75±4.42[a]	29.69±2.69[a]
	45	27.06±0.08[b]	10.43±2.22[a]	9.23±1.31[a]	29.00±0.83[a]
	135	26.43±0.64[b]	13.33±2.62[a]	13.19±2.25[a]	30.61±0.56[a]
Steamed					
GVRV	0	27.45±1.63[a]	11.77±1.35[a]	12.27±1.55[a]	29.91±2.34[a]
	45	26.67±0.68[b]	8.56±0.95[a]	16.61±3.28[a]	28.01±0.58[b]
	135	27.12±0.50[a]	11.70±1.71[a]	16.22±0.19[a]	29.62±0.92[a]
GCLV	0	24.83±0.26[b]	8.98±0.36[a]	0.87±0.28[b]	26.41±0.35[b]
	45	25.19±1.09[b]	10.28±2.78[a]	1.49±1.57[b]	27.24±1.35[b]
	135	24.02±0.63[b]	11.20±1.76[a]	3.57±1.43[b]	29.62±0.35[a]

Averages followed by the same letter, in the column, do not differ significantly, at a 5% probability level by Tukey Test. TA = Storage time (Days); C= Control; VR = Verê genotype- CL= Clevelândia genotype; GVRE= Crushed Verê genotype; GCLE= Crushed Clevelândia genotype; GVRV= Steamed Verê genotype; GCLV= Steamed Clevelândia genotype.

[1]) for jabuticaba and camucamu (*Plinia dubia)* (31.0 mg CE 100g^{-1}). Dessimoni-Pinto et al. (2011) used jabuticaba from Diamantina – MG, from whose peel, there was production of jelly (87.80 mg CE 100 g^{-1} fresh mass) and considered as rich in flavonoids.

Total anthocyanin content found in Clevelândia genotype peel powder differed statistically ($p<0.05$) from Verê genotype (Figure 2), during storage and between extraction methods. Clevelândia genotype have higher concentrations of anthocyanins and such color is related to the hydroxyl, methoxyl and glycol groups present in these structures (Lee et al., 2005). Such contents demonstrated that both genotypes contain elevated concentrations of this compound. Due to jabuticaba peel color (pigmented, dark purple color), it could be predicted (Santos and Meirelles, 2009; Veggi et al., 2011). a positive association between the Verê genotype's SSC was also observed in steam extraction with their anthocyanin values, showing lower SSC concentrations and lower concentrations of anthocyanins. Anthocyanins are rarely found free in plant, being connected to various sugars (Francis, 2000). Therefore, this method of extraction may have been responsible for smaller anthocyanin values due to loss of organic compounds by temperature process. Previous studies, which looked forward to testing tropical fruits that were not traditional in Brazil reported better results of anthocyanin content for jabuticaba Paulista (58.1 mg Cy-3-glicoside 100 g^{-1}) (Rufino et al., 2010). In recent work, Böger (2013) while analyzing different ethanoic extracts of jabuticaba peel, obtained values that were similar to those present in this work (27.02 and 60.32 mg Cy-3-glicoside 100g^{-1}), in same analytical conditions.

At 135 days, crushing extraction of Clevelândia genotype had lower levels in comparison with its early days. This degradation may occur by different mechanisms during storage, leading to formation of insoluble dimming products or colorless soluble products (Francis, 2000), but visually the samples showed no differences in this period.

CIELAB system attribute evaluation was performed due to the fact that Verê and Clevelândia genotype peels extracted by steam and crushing were subjected to dehydration under 70°C and stored at 0, 45 and 135 days (Table 2). Values of light coordinates (L*) vary from 0 to 100, where dark is close to 0 and light is close to 100. There was statistical difference in this parameter among fresh peels from Verê and Clevelândia with 23.46 and 20.25 light successively (in a 0 to 100 scale) determined by L* parameter. Where Verê's tone is closer to white, in other words it is lighter, if compared with Clevelândia. Color intensity close to Verê, as defined by C parameter was 10.18 and Clevelândia 4.89, showing another statistical difference, showing that Verê has a more intense color when fresh, but when dehydrated by steam and crushing methods, did not differ statistically until 135

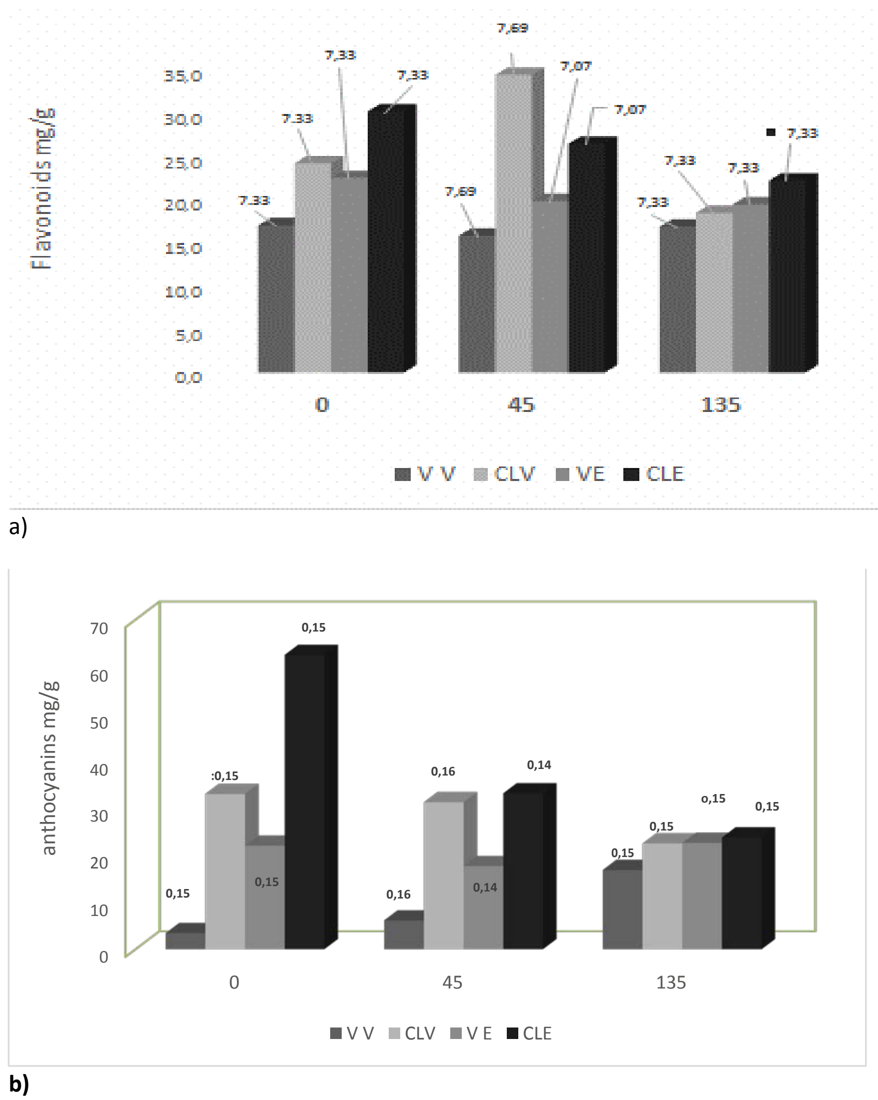

Figure 2. Bioactive compounds of jabuticaba peel powder after dehydration and 135 days storage. VV= Verê Steam; VE= Crushed Verê; CLV= Clevelândia Steam; CLE= Clevelândia Crushing.

storage days.

Tone parameter defined by H* parameter was 7.04 for Verê, however, for Clevelândia it was 171.13 which besides differing statistically, showed a quite elevated tone. Ariais (2000) observed similar results (180.58) in tomatoes (*Lycopersicon esculentum* cv. Laura) in different levels of maturation. But in general, none of the genotype peels showed loss in color tone, light intensity in any of the treatments, when dehydrated, but, regarding light (L*) and color tone of Verê dehydrated peels extracted by crushing and steam differed statistically as compared to Clevelândia, where Verê had a lighter peel.

H* parameter lower values in this same treatment (Verê/steam) demonstrated a reduction of fruit tone after dehydration, when compared with the same process applied to Clevelândia genotype. In a work performed with peppermint leaves, Gasparin et al. (2014) claimed that Chroma index (C*) indicates tone intensity or purity. If values are higher, color is more chromatic and brighter and when values are lower, the color is achromatic and opaque. However, authors presented higher values for fresh leaves, differing statistically from fresh leaves when subjected to drying, the opposite of what happened in this experiment.

According to Silva et al. (2007), ΔE values above 0.2-0.5 may express color differences in the two juxtaposed samples, with a very small perception. When ΔEab values are above 6.0, it is classified as a very big perception, according to DIN 6174 norm (1979), which establishes relationship with human eye. This way, ΔEab results shown in Table 2, demonstrate that all treatments, when compared with the control, both fresh and dry peels dehydrated and stored for both genotypes, showed color changes, which are noticeable by the human eye.

Conclusion

Vere is more acid and has higher ash content than Clevelândia genotype with is indicative of residual minerals in this product, thus adding value. Clevelândia genotype has higher SSC and is less acidic being more suitable for *in natura* consumption. Product humidity should remain lower than 14%, to increases its lifetime. The peel powder of both Jabuticaba genotypes are rich in flavonoids and steam extraction was more effective. Clevelândia genotype has higher amount of anthocyanins, but both genotypes showed high levels of this compound. The storage for 135 days demonstrated high levels of phenolic and anthocyanin compounds despite occurrence of losses. Color quality is enhanced when the peel is dehydrated with attractive pigmentation for blends in food. Both genotypes are suitable for food processing industries.

Conflict of Interests

The authors have not declared any conflict of interests.

REFFERENCES

Abe LT, Lajolo FM, Genovese MI (2012). Potential dietary sources of ellagic acid and other antioxidants among fruits consumed in Brazil: Jabuticaba (*Myrciaria jaboticaba* (Vell.) Berg). J. Sci. Food Agric. 9:1679-1687.

Alves SM, Silveira AM (2002). Estudo da secagem de tomates desidratados e não desidratados osmoticamente. Rev. Univ. Rural 21:21-30.

Araújo CRR (2011). Composição química, potencial antioxidante e hipolipidêmico da farinha da casca de Myrciaria cauliflora (jabuticaba). Dissertação - Mestrado em química. Universidade Federal dos Vales do Jequitinhonha e Mucuri, Diamantina 119p.

Arias R, Lee TC, Logendra L, Janes H (2000). Correlation of lycopene measured by HPLC with the L*, a*, b* color readings of a hydroponic tomato and the relationship or maturity with color and lycopene content. J. Agric. Food Chem. 48:1697-1702.

Barros RS, Finger FL, Magalhães MM (1996). Changes in nonstructural carbohydrates in developing fruit of *Myrciaria jaboticaba*. Sci. Hortic. 66:209-215.

Boari Lima AJ, Corrêa AD, Alves APC, Abreu CMP, Dantas BAM (2008). Caracterização do fruto jabuticaba (*Myrciaria cauliflora*) e de suas frações. Arch. Latinoam. Nutr. 58:416-421.

Boekel SV, Couto MAP, Ascheri, JLR, Srur AUOS, Lima ECS (2011). Elaboração de farinha mista extrusada de arroz, soja e resíduo de laranja-pêra como fonte de fibra alimentar. Magistra 23:243-251.

Böger BR (2013). Elaboração de sorvete adicionado de extrato de cascas de jabuticaba (Plinia cauliflora): avaliação de compostos bioativos. 81f. Trabalho de Conclusão de Curso (Graduação) – Curso Superior de Tecnologia de Alimentos. Universidade Tecnológica Federal do Paraná, Francisco Beltrão.

BRASIL (1978). Ministério da Saúde. Agência Nacional de Vigilância Sanitária. Resolução CNNPA nº. 12, de 24 de julho de. Regulamento técnico para produtos de cereais, amidos, farinhas e farelos. Diário Oficial da União, Brasília, DF, 24 jul.1978. Disponível em: <http://www.anvisa.gov.br>. Acesso em: 01 jul. 2015.

Chang C, Yang M, Wen H, Chern J (2002). Estimation of total flavonoid contente in própolis by two complementary colorimetric methods. J. Food Drug Anal. 10:178-182.

CIE (1995).Comission Internacional de L'éclairage, *Industria Colour-Difference Evaluation* Viena: Austria CIE 116.

Dessimoni-Pinto NAV, Moreira WA, Cardoso LM, Pantoja LA (2011). Jaboticaba peel for jelly preparation: an alternative technology. Ciência e Tecnologia de Alimentos, 31:864-869.

DIN (Deutsche Institut für Normung) (1979). DIN 6174. Farbmetrische Bestimmung Von Farbabständen bei Körperfarben nach der CIELAB-Formel, Beuth Verlag, Berlim und Köln.

Faroni LRA, Cordeiro IC, Alencar ER, Rozado AF, Alves WM (2006). Influência do conteúdo de umidade de colheita e temperatura de secagem na qualidade do feijão. Rev. Bras. Engen. Agríc. Amb. 10:148-154.

Fernandes AF, Pereira J, Germani R, Oiano-Neto J (2008). Efeito da substituição parcial da farinha de trigo por farinha de casca de batata (*Solanum tuberosum* Lineu). Ciênc. Tecnol. Alimen. 28:56-65.

Francis FJ (2000). Anthocyanins and betalains: composition and apllications. Cereal Foods Wordl, 45:208-213.

Gasparin PP, Alves, NCC, Christ D, Coelho SRM (2014). Qualidade de folhas e rendimento de óleo essencial em hortelã pimenta (*Mentha x Piperita* L.) submetida ao processo de secagem em secador de leito fixo. Rev. Bras. Plantas Med. 16:.

Instituto Adolfo Lutz (2008). Normas analíticas, métodos químicos e físicos para análise de alimentos. IAL, 3 ed. 1:53.

Lee J, Durst RW, Wrolstad RE (2005). Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH. J. AOAC. Int. 88:1-10.

Lenquiste SA, Batista AG, Silva Marineli R, Dragano NRV, Maróstica JRMR (2012). Freeze-dried jaboticaba peel added to high-fat diet increases HDL-cholesterol and improves insulin resistance in obese rats. Food Res. Int. 49:153-160.

Lima AJB, Duarte CA, Carvalho AAP, Patto ACM, Dantas-Barros AM (2008). Caracterização química das jabuticabas (*Myrciaria cauliflorara Berg*) e respectivas frações. Latino Am. Nutr. 58:416-421

Marquetti C (2014). Desenvolvimento e obtenção de farinha de casca de jabuticaba (Plinia cauliflora) para adição em biscoito tipo cookie. 116 f. Dissertação (Mestrado em Tecnologia de Alimentos) - Universidade Tecnológica Federal do Paraná, Londrina.

Rufino MSM, Alves RE, Brito ES, Pérez-Jiménez J, Sauracalixto F, Mancini-Filho J (2010). Bioactive compounds and antioxidant capacities of non-traditional tropical fruits from Brazil. Food Chem. 121:996-1002.

Santos DT, Meireles MAA (2009). Jabuticaba as a source of functional pigments. Pharmacogn. Rev. 3(5):127-132.

Sasso SAZ, Citadin I, Danner MA (2010). Propagação de Jabuticabeira por estaquia. Rev. Bras. Frutic. 32(2):577-583.

Silva PI, Stringheta PC, Teófilo RF, Oliveira IRN (2013). Parameter optimization for spray-drying microencapsulation of jaboticaba (*Myrciaria jaboticaba*) peel extracts using simultaneous analysis of responses. J. Food Engineer. 117:538-554.

Soares A, Freire-Júnior G, Siqueira RS (1992). Hygiene course and sanitation in the food industry (Handout). Rio de Janeiro, Embrapa – CTAA. 97 p.

Veggi PC, Santos DT, Meireles MAA (2011). Anthocyanin extraction from Jabuticaba (*Myrciaria cauliflora*) skins by different techniques: economic evaluation. Proced. Food Sci. 1:1725-1731.

academicJournals

Vol. 11(33), pp. 3162-3168, 18 August, 2016
DOI: 10.5897/AJAR2016.11361
Article Number: 26B086960050
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

The use of tannery sludge vermicomposting in corn crop irrigated with sewage treated wastewater

Aline Sueli de Lima Rodrigues[1], Fernando Godinho de Araújo[2], Wilson Mozena Leandro[3], Ivandilson Pessoa Pinto de Menezes[1], Caio César Oliveira Pereira[2] and Guilherme Malafaia[1]*

[1]Department of Biology, IF Goiano, Urutaí, Goiás, Brazil.
[2]Department of Agronomy, IF Goiano, Urutaí, Goiás, Brazil.
[3]Department of Agronomy, Universidade Federal de Goiás, Câmpus Samambaia, GO, Brazil.

Received 24 June, 2016; Accepted 26 July, 2016

The countless generation of waste (solid or liquid) by agro-industrial activities, has stimulated the development of researches aimed not only at the processing of these materials but also the possibility to take advantage of them in the agricultural environment. Therefore, this study aimed to evaluate the production of maize plants (*Zea mays* cultivar LG 6036) grown in pots containing Oxisol in addition to tannery sludge vermicompost and irrigated with treated wastewater from households. The treatments consisted of factorial 2x6 (two types of irrigation and six fertilization treatments) in a completely randomized design with five replications. At the end of the experiment, the following production components were evaluated: 100 grain weight (g) (13% moisture - dry basis), total number of grains per spike, total weight of grains per spike and total weight of the spike (g). It was observed that plants grown in soil increased with tannery sludge vermicomposting and irrigated with treated wastewater had higher yields, which indicates that these residues are important sources of nutrients for growing corn.

Key words: Agro-industrial waste, reuse of treated wastewater, waste recycling, sustainable agriculture.

INTRODUCTION

Currently, there is an increase in the development of researches aiming beyond treatment and the recovery of waste produced by agro-industrial activities. Environmental issues, in particular, have raised concerns and reflections, since the waste generated have the potential to cause environmental damage, if not properly treated (Kraemer, 2014). Therefore, in order to solve or minimize this issue, the reuse of this waste has emerged as an interesting and environmentally sustainable alternative since it can reduce the environmental problem, which is the disposal of these materials on the environment (Nunes et al., 2009).

Treated wastewater reuse for irrigation, landscape and surface or groundwater replenishment purposes is being widely implemented (Abourached et al., 2016; Fiorentino et al., 2016; Chen et al., 2016). Although, the reuse practice is accompanied by a number of benefits relating to the enhancement of water balances and soil nutrition

*Corresponding author. E-mail: guilherme.malafaia@ifgoiano.edu.br. Tel: +55 (64) 3461 1900 or +55 (64) 98401 7202.

by the nutrients existing in the treated effluents, a number of unanswered questions are still related to this practice (Fatta-Kassinos et al., 2011).

Besides the lack of knowledge in respect to possible elemental interactions that may influence the accumulation of heavy metals and other elements in the soil and the subsequent uptake by plants and crops, during the last several years, the technological progress in respect to analytical chromatographic methods has enabled the identification and quantitation of a number of organic xenobiotic compounds in treated wastewater. Therefore, it is now known that the effluents' remaining organic matter most usually expressed as chemical oxygen demand consists of a number of biorecalcitrant organic xenobiotic compounds including potential endocrine disrupting compounds (EDCs), pharmaceuticals, etc. It is also widely accepted that the currently applied treatment processes for urban wastewater abatement fail to completely remove such contaminants and this lead to their subsequent release in the terrestrial and aquatic environment through disposal and reuse applications. According to Fatta-Kassino et al. (2011), the number of studies focusing on the analysis and the toxicological assessment of such compounds in the environment is constantly increasing the aim being to bridge the various knowledge gaps associated with these issues. Thus, the existing knowledge in respect to the relevant existing legislation framework, the types of elements and chemicals of concern, the uptake of xenobiotic pollutants and also that of other neglected chemical elements along with their potential environmental interactions should be the focus of different studies.

According to Toze (2006), the reuse of water for agricultural irrigation is often viewed as a positive means of recycling water due to the potential large volumes of water that can be used. Recycled water can have the advantage of being a constant, reliable water source and reduces the amount of water extracted from the environment. In addition, in some cases, treatment requirements may need to be less than for water used in an urban environment due to less potential human contact. There are concerns and unknowns, however, about the impact of the quality of the recycled water, both on the crop itself and on the end users of the crops. Water quality issues that can create real or perceived problems in agriculture include nutrient and sodium concentrations, heavy metals, and the presence of contaminants such as human and animal pathogens, pharmaceuticals and endocrine disruptors (Toze, 2006). Thus, the minimization of human exposure to the practice of agricultural reuse is based on a set of mitigation measures that must be implemented by the authorities responsible for operating and monitoring systems for water recycling.

On the other hand, a generating activity of potentially toxic residue, very common in India and Brazil, refers to the bovine leather processing, through tanneries industries (Godecke et al., 2012). This brings several benefits in terms of jobs generation and income, however, they are faced with environmental problems, since the leather processing makes use of many potential polluting chemical (Godecke et al., 2012; Luersen et al., 2012). The problem becomes even worse when it was found that due to the great demand for products derived from tannery activity, large volumes of organic waste are generated (Godecke et al., 2012). Therefore, the agronomic use of these materials has been considered for reuse especially because most of these are compost of organic materials effective for fertilization and neutralization of acid soils (Godecke et al., 2012). However, the application of such natural wastes directly into the ground has caused controversy and widely varying results in different agricultural crops.

Thus, vermicomposting of waste tannery, biotechnological process that requires simple and low cost facilities, appears as an option for the recycling of this waste in the agricultural environment. As discussed by Vig et al. (2011), this process has been considered as a potential option in the hierarchy of integrated solid waste management, mainly because the unused solid waste can be transformed into noble organic compounds. According to Aquino et al. (1992), the use of vermicomposting in certain cultures may be more interesting than the use of natural waste, since vermicomposting can generate material with high agricultural potential.

Moreover, there are great demands for the use of treated wastewater coming from the domestic sewage produced daily. With the emergence of conflicts over water use and the fact that water consumption for irrigation is significant, the interest in the use of sewage to replace or complement the sources normally used for irrigation has increased.

As discussed by Leal et al. (2011), the prospects for irrigation in Brazil, with domestic treated wastewater, are promising considering the fact that agriculture plays an essential role in the economy, besides the fact that fresh water for irrigation of crops is scarce in some regions. Different studies have pointed to the potential use of this water in agriculture; this is due to the fact that the water has nutrients that are beneficial for plant growth (Andrade-Filho et al., 2013; Bonini et al., 2014; Leal et al., 2011; Silva et al. 2014). According to Hespanhol (2003), treated wastewaters from domestic sewage have nutrients whose content meets, if not all, at least most of the nutritional needs of plants in general.

Therefore, the present research aims to evaluate the development and productivity of maize in the soil with tannery sludge vermicomposting and irrigated with domestic treated wastewater. In addition, the authors sought to evaluate the nutritional quality of the crop by leaf analysis and the chemical impact of the combined use of such waste in the main characteristics of the soil.

Table 1. Main characteristics of the initial soil and tannery sludge vermicompost used in this study. Urutaí, GO, 2014.

Variables	Results		
	Soil	Vermicompost (Lc20)*	Vermicompost (Lp20)*
pH ($CaCl_2$)	5.30	8.8	8.8
N (%)	0.11	1.5	1.2
P (Melich – $mg.dm^{-3}$)	5.00	700.0	400.0
K ($mg.dm^{-3}$)	240.00	18,000.0	20,000.0
Ca ($cmolc.dm^{-3}$)	2.60	14.0	14.0
Mg ($cmolc.dm^{-3}$)	0.80	18.0	15.0
Ca + Mg ($cmolc.dm^{-3}$)	3.40	32.0	29.0
Al ($cmolc.dm^{-3}$)	0.00	0.0	0.0
H + Al ($cmolc.dm^{-3}$)	2.20	0.0	0.0
CTC ($cmolc.dm^{-3}$)	6.20	82.4	85.4
Na ($mg.dm^{-3}$)	8.00	1,000.0	1,200.0
Cu ($mg.dm^{-3}$)	2.50	5.0	2.9
Fe ($mg.dm^{-3}$)	63.00	244.0	122.0
Mn ($mg.dm^{-3}$)	47.00	68.0	55.0
Zn ($mg.dm^{-3}$)	4.40	36.0	39.0
Organic matter (%)	2.30	29.9	24.2
Sat Al (%)	0.00	0.0	0.0
Sat Base (%)	65.00	100.0	100.0
Ca/Mg (%)	3.30	0.8	0.9
Ca/CTC (%)	42.00	17.0	16.0
Mg/CTC (%)	13.00	22.0	18.0
K/CTC (%)	10.00	56.0	60.0
H + Al/CTC (%)	35.00	0.0	0.0
Clay (%)	27.00	-	-
Silt (%)	15.00	-	-
Sand (%)	58.00	-	-
Electrical conductivity ($\mu S.cm^{-3}$)	184.00	1,170.0	1,850.0
Total organic carbon (%)	1.30	17.3	14.0
Particle density ($g.cm^{-3}$)	2.45	-	-
Cr ($mg.dm^{-3}$)	<5,00	<5.0	<5.0

*Vermicompost (Lc20): tannery sludge vermicompost compost of 20% of liming tannery sludge and 80% of cattle manure. Vermicompost (LP20): tannery sludge vermicompost compost of 20% of primary tannery sludge and 80% of cattle manure.

MATERIALS AND METHODS

This research was conducted in a greenhouse, from April to August 2014, at the Experimental Station of the Instituto Federal Goiano (IF Goiano) - Campus Urutaí (GO, Brazil). The soil used in the experiment was taken from the surface layer, 0-20 cm, of an area next to the greenhouse, having been classified as Oxisol soil (Table 1).

The vermicomposting used were those produced from substrates compost of 20% liming and primary tannery sludge types, and 80% of cattle manure, which were produced as described by Malafaia et al. (2011). It is noteworthy that the liming sludge used in this research refers to the waste produced in the waxing phase of the skin and the primary sludge from the primary treatment to the tannery station industry. The grantor company for sludge treats the effluents generated in the bovine leather tanning stage, separately from other waste and produced effluents. Therefore, the tannery sludge used in this research did not contain the element Cr. Table 1 shows the characterization of such compounds done according to Tedesco et al. (1995).

The factorial arrangement of treatments consisted of a 2x6 (two types of irrigation and six fertilization treatments) in a completely randomized design (CRD) with five repetitions, making up a total of sixty experimental units. The following experimental units were established, irrigated with water supply (A) and with treated wastewater from households (R): (T1) Soil - control, without chemical fertilizer and without vermicomposting; (T2) Soil+ NPK; (T3) Soil + 20% of primary tannery sludge vermicomposting (VLp20); (T4) Soil + 20% of primary tannery sludge vermicomposting (VLp20) + P; (T5) Soil + 20% of liming tannery sludge vermicomposting (VLc20); and (T6) Soil + 20% of liming tannery sludge vermicomposting (VLc20) + P.

The dose of NPK used in treatments T2 (A) and T2 (R) was calculated based on the nutritional needs of the crop, the nutrient

Table 2. F test analysis of variance for 100 grains weight, total number of grains per spike, yield and total weight of the corn spike (*Zea mays* L. - LG 6036), depending on the type of water irrigation and fertilization treatments. Urutaí, GO, 2014.

Variation factors	Productivity (total weight of grains per spike)	100-grain weight (g)	Total number of grains per spike	Total weight of the spike (g)
Factor 1 (types of irrigation)	82.59**	7.62**	45.49**	107.92**
Factor 2 (treatments)	13.00**	4.80**	11.39**	15.65**
Interaction (factor 1 × factor 2)	8.60**	4.48**	6.12**	10.42**
CV (%)	19.38^{XX}	23.33**	22.33**	16.74**

*Significant at 1% probability; CV: coefficient of variation, expressed as percentage. Types of irrigation: water supply and treated wastewater from households. Treatments: Soil - control (without chemical fertilizer and without vermicompost); Soil + NPK; Soil + 20% of primary tannery sludge vermicompost (VLp20); Soil + 20% of primary tannery sludge vermicompost (VLp20) + P; Soil + 20% of liming tannery sludge vermicompost(VLc20); Soil + 20% of liming tannery sludge vermicompost (VLc20) + P.

concentrations in the soil and culture yield expectation of 10 Mg ha^{-1}, according to Souza and Lobato (2004). NPK sources were urea (CH_4N_2O), superphosphate (P_2O_5) and potassium chloride (K_2O), respectively. The doses of tannery sludge vermicomposting for the soil were calculated based on the concentration of K, high concentration element in vermicomposting used (Table 1) and the supply of 50 kg ha^{-1} K_2O at the base. The amount of 60 kg ha^{-1} K_2O was provided by fertilizing two plots of 30 kg ha^{-1}, at 40 and 60 days after sowing (DAS). Thus, the dose of liming sludge vermicomposting (VLc20) added to the soil corresponded to 6.1 Mg ha^{-1} and primary sludge vermicomposting (VLp20), 5.5 Mg ha^{-1}. It was not necessary to adjust the soil pH.

The soil previously mixed with vermicomposting (VLc20 and VLp20) and inputs were put in polyethylene vessels (volumetric capacity of 15 L) in a total of 12.5 kg. After the installation of the experimental units, the vessels were sown with three corn seed (*Zea mays* L.) cultivar LG 6036 (LG Seed®) and 15 days later, thinning was realized when one plant was kept by vessel. The phytosanitary treatment was performed when necessary and the nitrogen fertilization (total of 130 kg ha^{-1}) was performed on the surface, in two equal plots (65 kg ha^{-1}), at 40 and 60 DAS.

The water used in irrigation were those from the IF Goiano - Campus Urutaí water supply system, treated in a water treatment plant (WTP), and treated wastewater from a domestic wastewater stabilization pond, also located on the facilities of the institution. For the characterization of irrigation, water samples were collected monthly within the experimental period (n = 4) for evaluation of physical, chemical and physico-chemical parameters according to the methodology proposed by APHA (1997).

The management of crop irrigation was realized from an evaporimeter tank, developed by Salomão (2012), with a circular shape of 52 cm internal diameter and 24 cm height (internal), installed over a pallet of wood (height, 15 cm) and installed inside the greenhouse, between the treatments. To keep the soil water retention capacity in 70% (243.1 $mL.kg^{-1}$) during the experimental period, the volume of daily irrigation water was based on the vessel area to be irrigated (0.06 m^2) and crop evapotranspiration (ETc). It is noteworthy that the volume of water to be replaced was measured in a graduated cylinder. The soil water holding capacity (C100% = 347.4 $mL.kg^{-1}$) was determined by calculating the power of the soaking soil according to the methodology recommended by Embrapa (2007).

At the end of the trial period, the following production components was evaluated: 100 grain weight (g) (13% moisture - dry basis), total number of grains per spike, total weight of grains per spike and total weight of spike (g).

All the data were subjected to analysis of variance according to the factorial model (two-way ANOVA), and the factors were the treatments (six levels) and irrigation (two levels), with five replications. In cases of significant F, the Tukey test at 5% probability was performed. It is noteworthy that the analysis of variance was performed using the ASSISTAT software, version 7.7 beta (copy distributed for free).

RESULTS AND DISCUSSION

The results of analysis of variance indicated a high difference in the interaction between the sources of variation, "irrigation" and "treatments", for all yield components evaluated at the end of the experiment (100 grain weight (g), total number of grains per spike, total weight of grains per spike, and total spike weight (g) (Table 2). The interaction between the types of irrigation water × fertilization treatments for the production components that were evaluated can be seen in Table 3. Figure 1 shows images of ears of corn cobs for different experimental treatments.

The number of spike produced per plant was similar to the treatments (n = 1), and the total weight of grains per spike was taken as productivity, which is the total weight of grain produced per plant. For this variable, it was observed that the T6R treatment was higher than the T1R, T4R and T5R treatments and equal to the T2R and T3R treatments. It is noteworthy that the T3, T4, T5 and T6 treatments, irrigated with treated wastewater or not, had similar productivity with that observed in the T2 group (Table 3), which received chemical fertilizers. This indicates that the use of tannery sludge vermicomposting in the proportion used in this experiment can be an interesting alternative to reduce the use and cost of chemical fertilization, when it is aimed at producing maize.

Regarding the 100 grains weight, it was observed that there was difference only between the treatments irrigated with water supply (from T2A to T6A) when compared with its control (T1A) (Table 3). It is noted that the average number of grains for the last treatment was less than 100; therefore, the variable weight of 100 grains was estimated by a simple rule of three. Regarding the treatments irrigated with treated wastewater, no differences were identified (Table 3). Also, the grains produced have similar quality, state of maturity and sanity

Table 3. Mean values of interaction type of irrigation water x fertilization treatments for 100 grains weight, total number of grains per spike, yield and total weight of the corn spike (*Zea mays* L. - LG 6036). Urutaí, GO, 2014.

Types of irrigation	Treatments					
	Productivity (total grain weight per plant)					
	Soil (control)	Soil + NPK	Soil + VLp20	Soil + VLp20 + P	Soil + VLc20	Soil + VLc20 + P
Supply	011.83^{bC}	144.04^{bAB}	109.26^{bB}	173.08^{aA}	157.79^{aAB}	161.36^{bAB}
Treated wastewater	181.71^{aB}	194.81^{aAB}	211.58^{aAB}	183.79^{aB}	181.66^{aB}	249.61^{aA}
100-g weight (g)						
Supply	011.29^{bB}	041.24^{aA}	040.75a^{A}	041.77^{aA}	307.67^{aA}	039.34^{aA}
Treated wastewater	041.28^{aA}	046.53^{aA}	040.23a^{A}	037.94^{aA}	040.62^{aA}	043.94^{aA}
Number of grains per spike						
Supply	021.00^{bB}	370.80^{aA}	287.60^{bA}	431.80^{aA}	426.20^{aA}	419.40^{bA}
Treated wastewater	431.40^{aA}	417.80^{aA}	502.20^{aA}	479.60^{aA}	490.40^{aA}	580.00^{aA}
Total weight of the spike (g)						
Supply	020.23^{bC}	217.03b^{AB}	162.10^{bB}	255.61^{aA}	232.33^{bAB}	231.69^{bAB}
Treated wastewater	271.24^{aB}	282.63^{aAB}	300.90^{aAB}	277.89^{aAB}	284.37^{aAB}	349.89^{aA}

*Means followed by the same lower case letter in the column and capital on the line do not differ by Tukey test at 5% probability. VLp20: 20% of primary tannery sludge vermicompost; VLc20: 20% of liming tannery sludge vermicompost.

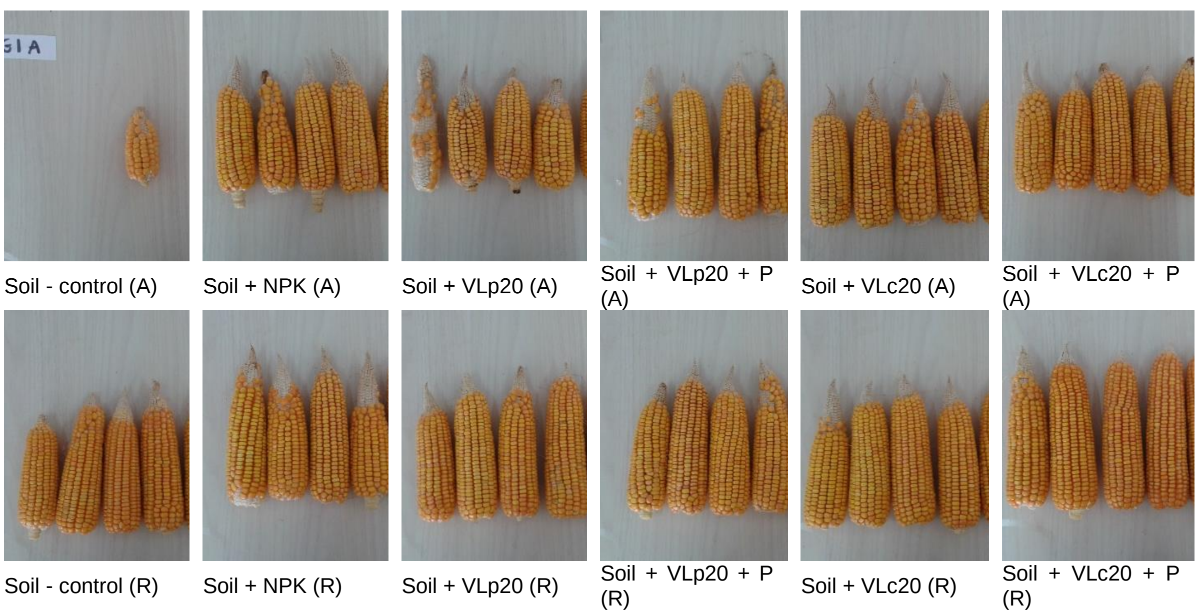

Figure 1. Images of corn cobs (*Zea mays* L. – LG 6036) produced by plants in the different experimental treatments.

with the treatments. As for the number of grains per spike produced by plants irrigated with the water supply, difference between the T2A to T6A treatments, compared to its control (T1A) was also observed. The treatments irrigated with treated wastewater also did not differ (Table 3).

Regarding the total weight of spikes produced by plants with treatments irrigated with the water supply, it was observed that the T2A to T6A treatments showed higher values for the variables as compared to their control, T1A. Also, the T3A, T4A, T5A and T6A treatments were similar to the treatment that received mineral fertilizers, T2A, (Table 3). Furthermore, with regards to the treatments irrigated with water supply, all the groups irrigated with treated wastewater, except T4R treatment, produced spikes with the highest total weight (Table 3).

The data obtained in this study, regarding the yield components evaluated, also allow us to infer that the combined use of tannery sludge vermicomposting and irrigation with domestic treated wastewater can be an interesting alternative for agricultural practice that reduces the amount of chemical fertilizer used in maize crops, embedding environmental benefits and human health.

Previous studies, such as the works by Panoras et al. (2004), Azevedo et al. (2007) and Costa et al. (2012) showed that the use of treated wastewater from sewage provides greater or similar productivity to maize cultivation held in conditions of high chemical fertilization. Similarly, studies by Chamle et al. (2006), Kalantari et al. (2010), Tejada and Benítez (2011), Manyuchi et al. (2013) and, more recently, Chamle (2014) and Pandurang (2014) evaluated the impact of the use of vermin-composting in maize and also showed positive effects on the crop. However, no study to date, had evaluated the effect of combined use of treated wastewater with tannery sludge vermicomposting on production parameters.

Conclusion

Based on the results and according to the experimental conditions, it can be concluded that both the tannery sludge vermicomposting and the treated wastewater from households, are important sources of nutrients for the cultivation of maize and helps in providing conditions that provide improved productivity in maize.

Conflict of interest

The authors have not declared any conflict of interest

REFERENCES

Abourached C, English MJ, Liu H (2016). Wastewater treatment by Microbial Fuel Cell (MFC) prior irrigation water reuse. J. Cleaner Product 137(20):144-149.

Andrade-Filho JA, Sousa ON, Dias NS, Nascimento IB, Medeiros JF, Cosme, CR (2013). Atributos químicos de solo fertirrigado com água residuária no semiárido brasileiro. Irriga. 18:661-674.

APHA (American Public Health Association). (1997). Standard methods for the examination of water and wastewater. 20. ed. New York: APHA, AWWA, WPCR. 1194 p.

Aquino AM, Almeida DL, Silva, VF (1992). Utilização de minhocas na estabilização de resíduos orgânicos: vermicompostagem. Rio de Janeiro: Embrapa/CNPBS 12 p.

Azevedo MRQA, Konig A, Beltrão NEM, Azevedo CAV, Tavares TL, Soares FAL (2007). Efeito da irrigação com água residuária tratada sobre a produção de milho forrageiro. Agrária 2:63-68.

Bonini MA, Sato LM, Bastos RG, Souza CF (2014). Alterações nos atributos químicos e físicos de um Latossolo Vermelho irrigado com água residuária e vinhaça. Rev. Biociênc. 20:56-63.

Chamle DR, Mogle U, Jadhav B (2006). Effect of vermicompost on chlorophyll content, leaf area yield of maize. Geobios. 33:334-336.

Chamle DR (2014). Effect of weed vermicomposts on maize. Adv. Res. Pharm. Biol. 4:622-625.

Chen A, Liang H, Chen T, Yang W, Ding C (2016). Influence of long-term irrigation with treated papermaking wastewater on soil ecosystem of a full-scale managed reed wetland. J. Soils Seed. 16(4):1352-1359.

Costa MS, Costa ZVB, Alves SMC, Neto MF, Marinho MJC. (2012). Avaliação nutricional do milho cultivado com diferentes doses de efluente doméstico tratado. Irriga 1:12-26.

Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA) (2007). Nutrição e Adubação do Milho. Available at: http://www.cnpms.embrapa.br/milho/deficiencia/defic6.html.

Fatta-Kassinos D, Kalavrouziotis IK, Koukoulakis PH, Vasquez MI. (2011). The risks associated with wastewater reuse and xenobiotics in the agroecological environment. Sci. Total Environ. 409(19):3555-3563.

Fiorentino C, Mancini M, Luccarini L (2016). Urban wastewater treatment plant provided with tertiary finishing lagoons: management and reclamation for irrigation reuse. J. Chem. Tech. Biotechnol. 91:1615-1622.

Godecke MV, Rodrigues MAS, Naime RH (2012). Resíduos de curtume: estudo das tendências de pesquisa. Reget. 7:1357-1378.

Kalantari S, Hatami S, Ardalan MM, Alikhani HA, Sharafa M (2010). The effect of compost and vermicompost of yard leaf manure on growth of corn. Afr. J. Agric. Res. 5:1317-1323.

Kraemer MEP (2014). A questão ambiental e os resíduos industriais. http://www.amda.org.br/imgs/up/Artigo_25.pdf

Leal RMP, Fonseca AF, Herpin U, Melfi AJ (2011). Agricultural utilization of treated sewage effluent: experience from Brazil. Israel J. Plant. Sci. 59:235-248.

Luersen LH, Shultz G, Rempel C. (2012). Reuso do efluente tratado na indústria curtumeira. Tecnológica 16:5-10.

Malafaia G, Jordão CR, Araújo FG, Leandro WM, Rodrigues ASL (2015). *Vermicompostagem de lodo de curtume em associação com esterco bovino utilizando* Eisenia foetida*.* Eng. Sanit. Ambient. 20(4):709-716.

Manyuchi MM, Kadzungura L, Phiri A, Muredzi P (2013). Effect of vermicompost, vermiwash and application time on *Zea mays* growth. Int. J. Sci. Eng. Technol. 2:638-641.

Nunes PMP, Smolarek FSF, Kaminski GAT, Fin MT, Zanin SMW, Miguel MD, Miguel OG (2009). A importância do aproveitamento dos resíduos industriais da semente de *Citrus*. Rev. Visão. 10:97-110.

Pandurang MU (2014). Efficacy of weed vermicompost and chemical fertilizer on yield, morpho-physiological and biochemical investigations of Maize. Int. J. Sci. Eng. Res. 2:19-22.

Panoras A, Evgenidis G, Bladenopoulou S, Melidis V, Doitsinis A, Samaras I, Zdragkas A, Marsi T. (2004). Corn irrigation with reclaimed municipal wastewater. Global Nest. J. 5:47-54.

Salomão LC (2012). Calibração de tanques evaporímetros de baixo custo sob diferentes diâmetros em ambiente protegido. 2012. 74f. Thesis (Doutorado em Agronomia)-Faculdade de Ciências Agronômicas da Universidade Estadual Paulista “Júlio de Mesquita Filho”, Botucatu. Available at: http://acervodigital.unesp.br/handle/unesp/157180?locale=pt_BR

Silva LL, Carvalho CM, Souza RPF, Feitosa HO, Feitosa SO, Filho RRG (2014). Utilização de efluentes domésticos no crescimento da pimenta (*Capsicumchinense*), cultivar tequila bode vermelha. Agropec. Técnica 35(1):121-133.

Souza DMG, Lobato E (2004). Calagem e adubação para culturas anuais e semiperenes. In: Souza DMG, Lobato E (Ed.). Cerrado: correção do solo e adubação. 2. ed. Planaltina: Embrapa Cerrado. pp. 283-315.

Tedesco MJ, Gianello C, Bissani C, Bohnen H, Volkweiss SJ (1995). Análise de solo, plantas e outros materiais. 2. ed. Porto Alegre: Universidade Federal do Rio Grande do Sul. 174p.

Tejada M, Benífez C (2011). Organic amendment based on vermicompost and compost: differences on soil properties and maize yield. Waste Manage. Res. 29:1185-1196.

Toze S (2006). Reuse of effluent water-benefits and risks. Agric. Water Manage. 80(1-3):147-159.

Vig AP, Singh J, Wani SH, Dhaliwal SS (2011). Vermicomposting of tannery sludge mixed with cattle dung into valuable manure using earthworm *Eisenia fetida* (Savigny). Bioresour. Technol. 102:7941-7945.

academicJournals

Vol. 11(33), pp. 3169-3177, 18 August, 2016
DOI: 10.5897/AJAR2016.11160
Article Number: D31747C60047
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Phenotypic traits detect genetic variability in Okra (*Abelmoschus esculentus*. L. Moench)

Aaron T. Asare[1]*, Elvis Asare-Bediako[2], Faustina Agyarko[2], Kingsley Taah[2] and Edmund O. Osei[3]

[1]Department of Molecular Biology and Biotechnology, School of Biological Sciences, CANS, UCC, Cape Coast, Ghana.
[2]Department of Crop Science, School of Agriculture, CANS, UCC, Cape Coast, Ghana.
[3]Plant Genetic Resources Institute (PGRRI), Bunso, Ghana.

Received 28 April, 2016; Accepted 16 June, 2016

There is low production of okra in Ghana due to lack of improved varieties and biotic constraints. This study was conducted to characterize okra genotypes to predict genetic variation in the crop. Field trial was conducted to determine genetic variability in 21 okra genotypes. The experiment was based on the randomized complete block design (RCBD) involving planting distance of 0.6 × 0.6 m. Thirty-one quantitative and qualitative data were used to generate a dendrogram. Variations in leaf shape, leaf rib colour, petiole colour, petal colour, colour of the darkest ridges and stem colour were distinctive among the okra genotypes. The mean plant height, canopy diameter, leaf length and breadth, petiole length, internode length, number of branches, days to 50% flowering and fruit yield differed significantly ($p \leq 0.05$) among the 21 okra genotypes. These were discriminated into three clusters in a dendrogram with GH3731 as the most diverse. UCCC1, UCCC2, UCCC3, UCCC4 and UCCC5 appeared genetically similar with low fruit yield but early maturity. However, GH5332 had a significantly ($p \leq 0.05$) the highest fruit yield of 11.88 t ha^{-1} but late maturing. UCCC5 or similar genotypes with early maturity trait can be hybridized with GH5332 to improve the yield and earliness.

Key words: Breeding, germplasm, genotypes, genetic diversity and hybridization.

INTRODUCTION

Okra (*Abelmoschus esculentus* L. Moench) of the family Malvaceae, is an important and widely cultivated annual crop in both the tropical and sub-tropical regions of the world (Eshiet and Brisibe, 2015; Ali et al., 2014). It is a vegetable rich in organic and inorganic nutrients that sustain human health and as feed for animals (Chattopadhyay et al., 2011; Ofoefule, 2001; Rahman et al., 2012; Wamanda, 2007; Siesmonsma and Kouame, 2004; Saifullah and Rabbani, 2009). In Ghana, okra is often consumed in the diet by both children and adults in

*Corresponding author. E-mail: aasare@ucc.edu.gh. Tel: +233 244954045.

both rural and urban communities. However, the yield of this crop is low due to lack of improved varieties, biotic and abiotic stresses. Yield potential of 2000 to 3000 kg ha^{-1} has been reported for Okra (MoFA, 2007), depending on the cultivar, harvesting frequency and period for harvesting (Cudjoe et al*.,* 2005). However, actual yields of okra are usually low and also decreased over the years in Ghana, in spite of its economic importance and health benefits (Asare-Bediako et al., 2014).

The development of new varieties with better adaptation and yield potential are crucial for sustainable production of okra. Genetic variation in okra is a necessary requirement to improve the crop. Omonhinmin and Osawaru (2005) reported that high degree of wide morphological variation was found among accessions of okra, especially in West African type. There are numerous cultivars of okra with varied plant height, degree of branching and pigmentation of the various parts, period of maturity, and pod shape and size (AdeOluwa and Kehinde, 2011). In addition, Bisht et al. (1995) observed that pigmentation and pubescence of stem, leaf, pods and seeds were important components of variability in okra germplasm.

Various types of okra in Ghana are cultivated in the savannah and forest agro-ecological zones that require assessment of their genotypes. Ahiakpa et al. (2013) has done some morphological characterization of okra in Ghana but lacked collections from the central region and inclusion of exotic genotypes. The current work considered collections from the central region and other regions of Ghana as well as Togo to enhance assessment of fully harness variability in the germplasm for breeding and conservation. Indeed, genetic variation may serve as recipe for controlled hybridization to improve the crop. Assessment of variable phenotypic traits of okra would be useful in predicting genetic diversity towards molecular characterization to establish the genetic structure of okra in Ghana. This will facilitate routine breeding and germplasm conservation of the crop. The objective of the current work was to explore phenotypic characteristics to predict genetic variability among 21 okra genotypes in Ghana and to establish basis for molecular characterization.

MATERIALS AND METHODS

Experimental site

The experiment was conducted at the Teaching and Research Farm of the School of Agriculture, at University of Cape Coast during the 2015 major crop season (June to October). This site is located within the coastal savannah vegetation zone, with Acrisol soil type and is a highly endemic site for viral diseases and flea beetle infestation. The area has a bi-modal rainy season from May to June and August to October with an annual rainfall ranging between 750 and 1000 mm and temperatures ranging between 23.2 and 33.2°C with an annual mean of 27.6°C (Owusu-Sekyere et al., 2011).

Plant

Twenty-one genotypes of okra (both landraces and improved) were used for the study (Table 1). These comprised of fifteen accessions from Plant Genetic Resource Research Institute (PGRR1) at Bunso (wide collections from regions of Ghana and Togo), four farmer varieties, a land race and an improved variety (Asontem) from the Central Region of Ghana. The local names and sources of 21 okra genotypes are shown in Table 1.

Field experiment

The randomized complete block design (RCBD) with twenty-one genotypes of okra was sown in four replications. A total land area of 1344 m^2 (84 × 16 m) was ploughed and harrowed to render the soil loose. It was then divided into four blocks and each block was further divided into 21 plots, with each plot measuring 3 × 3 m. A distance of 1.0 m was left as walkway between the blocks and 1 m between the plots. Planting was done in June, 2015. The 21 okra genotypes were sown directly at three seeds per hole at a planting distance of 0.6 × 0.6 m and a planting depth of not more than 0.5 cm. The seedlings were later thinned out leaving two seedlings per hill. Weed control was done as necessary using herbicides and a hoe (manual weeding). NPK fertilizer (15:15:15) was applied at a rate of 250 kg ha^{-1}. Watering was done when necessary using sprinklers.

Data collection and analysis

The quantitative and qualitative data were collected based on the International Plant Genetic Resource Institute (IPGRI, 1991) okra descriptor list and adopted the procedure by Ahiakpa et al. (2013) and Nwangburuka et al. (2011) with some modifications (Table 2). The quantitative data including plant height, canopy diameter, leaf length, breadth, and stem base diameter at first flowering stage and petiole length, fruit length and fruit girth were obtained using meter rule or tape measure. The number of branches and fruits per plant were counted and fresh weight of matured fruits were determined by electronic balance (Radwag, WPT 12C1, Poland). The qualitative parameters were determined by visual estimation and rated (Table 1).

Data on plant growth and yield parameters were subjected to one-way analysis of variance (ANOVA) to determine significant differences among the 21 okra genotypes. The means were separated by the least significant difference method, using GenStat Discovery version 4 (VSN International). The GenStat or Minitab 15 statistical software was used for all computations. The Pearson's correlation coefficients were estimated for the growth and yield parameters. The Eigen-vectors and factor scores were used respectively to measure the relative discriminative power of the PC-axes and their associated characters. The data involving 31 parameters (Table 2) were analyzed with the PowerMarker version 3.5 and the dendrogram generated in Molecular Evolutionary Genetics Analysis version 4 (MEGA4) to determine genetic relatedness among the okra genotypes (Tamura et al. 2007).

RESULTS AND DISCUSSION

Qualitative and quantitative characteristic variations exist among the 21 okra genotypes. Variations in matured leaf colour, leaf shape, leaf rib colour, petiole colour, petal colour, colour of the darkest ridges and stem colour were

Table 1. Sources of okra genotypes used for the study.

Accession number	Accession name	Country of origin (Location)
UCCC2	Odumase	Ghana (Fosu Odumase)
UCCC3	Antado	Ghana (Antado-KEEA)
UCCC4	Asontem	Ghana (Assin Fosu)
GH2026	Manshior	Togo
GH2052	Fetri (Ewe)	Togo
GH2057	Fetri	Togo
GH2063	Fetri	Togo
GH3731	Krotetenye	Ghana (Abortia Junction)
GH3734	Fetri	Ghana (Kpogadzi)
GH3760	Nkruma	Ghana (Nsapor)
GH4374	Nkruma	Ghana (Duabone No.1)
GH5302	Pɛbrɛnkruma	Ghana (Ayiogbe)
GH5321	-	Ghana (Pinihi)
GH5332	Bropo Asontem	Ghana (Fententaa)
GH5786	Tuagya	Ghana (Koranten)
GH5793	Ogye abatan	Ghana (Asikasu)
UCCC5	Kakumdo	Ghana (Kakumdo)
GH6105	Asontem	Ghana (Mankessim)
GH6211	Nkrumah	Ghana (Ashiaman)
UCCC6	UCC Campus	Ghana (UCC-Cape Coast)
UCCC1	Avalavi	Ghana (Assin Akonfodi)

distinctive differentiation characters. In addition, differences in flowering span, fruit colour, fruit shape and number of ridges per fruit, pubescence, position of fruits on main stem and branching position at main stem were evident among the okra genotypes (Table 3).

The mean plant height, canopy diameter, leaf length and breadth, petiole length, internode length and number of branches as well as days to first flowering, 50% flowering and fruit yield differed significantly ($p < 0.05$) among the 21 okra genotypes. However, the stem diameter did not show significant ($p > 0.05$) variation among the okra genotypes. The highest average plant height of 68.95 cm was noted for UCCC3 and the least plant height of 28.72 cm was for GH4374 (Table 4). Plant height at flowering and fruiting are of particular interest for breeding programmes, because the presence of plants with tall and thin stems will increase the rate of lodging near harvesting and this could lead to loss of dry matter and subsequent decrease in fruit yield (Esthiet and Brisibe, 2015). In fact, Verma (1993), Ariyo et al. (1987) and Perdosa (1983) intimated that plant height is controlled by genetic factors and is closely associated with number of flowering node, average fruits per plant and number of internodes.

The leaves of okra serve as the main sites for photosynthesis, an increase or a decrease in their size could affect production of assimilates in the crop. Larger size leaves in any okra genotype may have higher ability to intercept solar radiation to assume higher photosynthetic capacity, which may enhance growth and crop yield. GH3734 had the widest canopy diameter of 100.86 cm compared to the least of UCCC5 (59.14 cm). The highest leaf length of 21.82 cm was produced by GH3734 and the lowest of 14.84 cm was associated with UCCC5. The mean leaf breadth of 29.55 cm was the highest observed for GH3760 compared to the least average leaf breadth of 19.89 cm for UCCC5 (Table 4). According to Ahiakpa (2013), an increased leaf area index and a resultant higher fraction of intercepted radiation and its utilization efficiency may increase crop yield. Significant ($p < 0.05$) correlations were observed between leaf length and canopy diameter ($r = 0.72$), breadth and canopy diameter ($r = 0.53$), leaf length and breadth ($r = 0.65$), petiole length and canopy diameter ($r = 0.49$) and stem diameter and canopy diameter ($r = 0.70$) in the okra germplasm could be determinants for plant vigour and yield indicators. GH2026 had the longest internodes of 21.53 cm per plant compared with the shortest for UCCC4 (14.69 cm per plant).

The mean petiole length of 20.64 cm was the highest for GH2052 compared with that of the lowest average petiole length of 14.29 cm for UCCC5. GH2026 had highest mean internodes (21.53 cm) and number of branches (5), respectively. However, GH2057, GH2063, GH4372 and GH5793 had the least number of 2 branches per plant. Variations in petiole length, leaf size,

Table 2. Rating of morphological characters of the okra genotypes used for the study.

Character	Rating/Estimation
Seed colour (SC)	1 = Dark, 2 = Black, 3 = Whitish to dark, 4 = purple to black
Seed Shape (SSh)	1 = Roundness, 2 = Kidney, 3 = Shperical
Seed size (SS)	1 = Small, 2 = medium, 3 = Large
Branching position at main stem (BPMS)	1 = UOA- Unique orthotrop axis, 2 = DBO- densely branched all over, 3 = DBB- densely branched base
Mature leaf colour (MLS)	1 = Green, 2 = Green + red veins
Leaf shape (LSH)	From type 1 to 11
Length of Branches (LBr)	0 = No branches, 1 = branches rarely > 10cm
Leaf rib colour (LRC)	1 = Green, 2 = Green + red veins
Petiole colour (Ptc)	1 = Green, 2 = greenish-red, 3 = purple
Petal colour (PC)	1 = 1= Golden yellow, 2 = yellow
Colour of the darkest ridges (CDR)	1 = light, 2 = dark, 3 = light to dark
Stem colour (StC)	1 = Green, 2 = Green + purple tinge, 3 = purple
Flowering span (FSp)	1 = Single flowering, 2 = grouped flowering
Fruit colour (FC)	1 = Green, 2 = green + red spots, 3 = dark green-black, 4= green-yellow, 5 =purple
Fruit pubescence (FP)	1 = Smooth, 2 = little rough, 3 = downy + hairs
Fruit shape (FSh)	From type 1 to 15
Number of ridges/fruit (NR)	1 = 0, 2 = 5-12, 3 = 15 ridges
Position of fruit on main stem (PFMS)	1= intermediate, 2 = slightly falling, 3 = horizontal, 4 = erect, 5 = drooping
Plant height (PH)	-
Canopy diameter (CD)	-
Leaf length (LL)	-
Leaf breadth (LB)	-
Stem diameter (SD)	-
Petiole length (PL)	-
Internode length (IN)	-
Number of branches (NBr)	-
Fruit length (FL)	-
Fruit girth (FG)	-
Number of fruits per plant (NF)	-
Weight of fruits (WF)	-
Days to first flower (DF)	-
Days to 50 % flowering (DFF)	-

canopy diameter, number of branches and stem diameter may have implications for crop yield and stability to control lodging.

According to Ariyo and Odulaja (1991), variability in okra germplasm is more prominent in days to flowering, plant height and various fruit characteristics and these traits could be important in differentiating varieties of *A. esculentus*. Similarly, in the current study, fruit length, girth and weight as well as the days to first flowering and days to 50% flowering differ significantly ($p < 0.05$) among the 21 genotypes of okra (Table 5). However, UCCC1, UCCC2, UCCC3, UCCC4, and UCCC5 were very similar in early flowering and days to 50% maturity as well as average fruit number, weight and size. Generally, all okra genotypes with high vegetative growth delayed flowering and maturity. UCCC5 was first to flower at 47 days and attained 50% flowering at 53 days, respectively after sowing seeds, which were significantly early compared to others. On the contrary UCCC6 was very late to first flower at 139 days and 50% flowering at 141 days, which were significantly ($p < 0.05$) different from all the other okra genotypes.

In this study, the significantly ($p < 0.05$) high yielding okra genotype, GH5332, produced 20 fruits per plant, with the highest fruit weight of 11.88 t ha^{-1}, which compared well with the size of the fruits (mean fruit length of 14.2 cm, girth 20 mm, and weight of fruit per plant of 21.6 g) among the okra genotypes. However, GH5332 is late maturing with 50% flowering at 101 days. Indeed, Esthiet and Brisibe (2015) reported that fruit length, pod

Table 3. Qualitative parameters of 21 Okra genotypes

Genotype	SC	SSh	SS	BPMS	MLC	LSh	LBr	LRC	PtC	PC	CDR	StC	NES	FSp	FC	FP	FSh	NR/F	PFMS
GH2026	1	1	2	3	2	3	1	1	3	2	1	3	1	1	1	2	4	2	3
UCCC2	1	1	2	1	1	2	0	1	1	2	1	1	1	1	1	3	3	2	1
UCCC4	1	3	1	1	1	9	0	1	1	2	1	1	2	1	1	2	8	2	1
GH3760	3	2	3	2	2	6	0	1	3	2	1	2	1	2	1	1	7	1	3
GH 2057	3	1	3	2	2	1	1	1	1	2	1	1	1	2	1	1	4	2	3
GH 6105	3	3	3	2	1	1	0	1	3	2	1	1	2	2	3	1	7	1	3
UCCC3	1	1	2	1	1	9	0	1	1	2	1	1	1	1	1	2	3	2	2
GH 5332	3	1	3	3	2	1	1	2	3	2	1	2	2	1	5	1	7	0	5
UCCC6	2	2	3	2	2	3	0	1	1	2	1	1	1	1	4	1	3	2	5
GH5302	2	2	3	2	2	3	0	1	3	2	1	1	2	2	5	1	4	2	3
GH5786	3	3	2	2	2	1	1	1	3	2	1	2	2	2	1	2	4	2	2
GH4374	1	1	3	3	2	3	1	1	3	2	1	2	2	2	1	1	12	2	4
GH3731	2	3	1	1	2	3	0	2	3	1	2	3	2	2	5	1	8	1	5
UCCC1	1	1	2	2	1	6	0	1	1	2	1	1	2	1	1	2	3	2	4
GH 3734	1	2	1	1	1	4	0	1	1	2	1	1	2	1	1	3	12	2	4
GH 5321	3	1	2	2	1	3	1	1	2	2	1	1	2	1	1	2	15	2	1
GH 5793	2	2	3	3	2	3	1	1	2	2	1	1	1	2	1	1	3	2	5
GH 2063	1	2	3	3	2	1	1	1	3	2	1	2	2	2	1	3	3	2	1
GH 6211	1	1	2	2	2	3	0	1	3	2	1	1	1	1	1	3	12	2	4
GH 2057	3	1	3	2	2	1	1	1	1	2	2	1	2	2	5	3	3	2	1
UCCC5	1	1	3	2	2	3	1	1	3	1	1	2	2	2	1	2	3	2	3

Seed colour (SC), Seed Shape (SSh), Seed size (SS), Branching position at main stem (BPMS), Mature leaf colour (MLC), Leaf shape (LSH), Length of Branches (LBr), Leaf rib colour (LRC), Petiole colour (Ptc), Petal colour (PC), Colour of the darkest ridges (CDR), Stem colour (StC), Number of epicalyx segments (NES) Flowering span (FSp), Fruit colour (FC), Fruit pubescence (FP), Fruit shape (FSh), Number of ridges per fruit (NR) and Position of fruit on main stem (PFMS).

number and pod weight are the most important determinants of production or yield in okra. It has been suggested that the number of days and plant height at flowering are controlled by the same genetic variables (Choudhary et al., 2006; Hussain et al., 2006).

It is critical to consider early maturity in the phase of erratic rainfall as essential trait to complement yield for hybridization to produce climate-smart okra genotypes. Therefore, UCCC5 with very early flowering and fruiting traits can be hybridized with the high yielding but late maturing genotypes of GH5332 to improve the crop. A successful cross between unrelated varieties may result into an array of elite genotypes from which advantageous agronomic line may be selected (Ali et al., 2014).

The variation in the quantitative characteristics which accounted for the total variance includes number of fruits per plant, mean plant height, canopy diameter, leaf length, breadth, stem diameter, petiole length, internode length, and number of branches. The proportion contributed by each quantitative variable to determine the total variation within each Principal Component (PC) axis is shown in Table 6. The variations in the quantitative characters contributed significantly

Table 4. Variation in growth characteristics of 21 okra genotypes.

Accessions	PH (cm)	CD (cm)	LL (cm)	LB (cm)	SD (cm)	PL (cm)	IN (cm)	NBr
GH2026	30.47	77.05	17.96	23.92	3.62	19.44	21.53	5
GH2052	17.96	84.57	17.53	27.29	3.3	20.64	18.83	3
GH2057	40.5	85.7	20.57	29.36	3.93	19.5	17.89	2
GH2063	31.61	90.36	19.97	26.39	3.51	20.15	15.89	2
GH3731	41.88	74.44	17.92	22.5	3.44	17.06	18.09	3
GH3734	60.98	100.86	21.82	26.68	4.11	22.16	19.42	4
GH3760	37.08	83.32	20.44	29.55	3.28	21.56	18.71	3
GH4374	28.72	79.08	17.64	23.02	4.21	18.2	17.56	2
GH5302	34.38	87.73	20.59	27.3	3.92	20.46	20.5	3
GH5321	47.32	77.03	18.13	25.47	4.43	19.53	20.32	3
GH5332	42.61	76.41	18.06	25.88	3.23	19.04	16.78	3
GH5786	34.51	86.09	20.52	27.68	3.75	21.57	19.47	3
GH5793	37.24	85.75	20.72	28.46	3.31	17.6	17.2	2
GH6105	42.52	93.49	20.39	24.54	3.9	20.46	18.36	3
GH6211	59.81	79.07	21.03	21.9	3.44	15.47	20.36	3
UCCC1	76.65	70.85	17.11	22.36	2.88	17.82	19.93	3
UCCC2	57.64	75.01	17.35	19.64	3.05	15.76	20.11	3
UCCC3	68.95	79.15	17.59	23.02	3.08	18.48	19.19	3
UCCC4	56.63	81.39	17.58	22.68	3.24	18.25	14.69	3
UCCC5	40.64	59.14	14.84	19.89	2.64	14.29	17.41	3
UCCC6	35.67	87.93	20.47	28.88	3.61	20.29	14.97	3
SE	14.3	20.51	4.97	7.03	2.01	7.15	6.31	2.02
Lsd	6.62	9.49	2.3	3.25	-	3.31	2.92	0.94

Plant height (PH), Canopy diameter (CD), Leaf length (LL), Leaf breadth (LB), Stem diameter (SD), Petiole length (PL), Internode length (IN and Number of branches (NBr).

(Eigen vector ≥ 0.2) to the variation within each of the four PC-axes as 38.00, 14.90, 12.40 and 11.70% for PC1, PC2, PC3 and PC4, respectively. The cumulative proportion of variation explained by the first four PC-axes, 77.00% (Table 6) compared well with observations made by Campos et al. (2005) and Ogunbayo et al. (2005) that the PC-axes contributed 76.62 and 64.5% variations, respectively. Similarly, Ahiakpa et al. (2013) reported that the first four PC-axis contributed 82.97% of the variations in okra. The remaining six axes in the current study accounted for only 23.00% of the total variation. Indeed, canopy diameter, leaf length, breadth, stem diameter and petiole length contributed to the variation in PC1. Plant height, length of internodes and number of branches accounted for the variations observed in PC2 and fruit per plant as well as plant height contributed to the variations in PC4. These variations may suggest the existence of genetic diversity in okra that can be harnessed to improve the crop. Similar observation was made by Yonas et al. (2014).

The 21 okra genotypes were distinguished into 3 main clusters (I, II and III) in the dendrogram at 43% genetic dissimilarity based on 31 quantitative and qualitative morphological characters (Figure 1). All the clusters were made up of varied sub-clusters with the exception of Cluster II, which had a single okra genotype (4.8%) involving GH3731, but the most diverse of all. However, cluster III made of 33.3% of the 21 okra genotypes appeared more closely related, including GH3734, GH6211, UCCC1, UCCC2, UCCC3, UCCC4 and UCCC5, which may suggest genetic similarity. The remaining 61.9% of the okra genotypes were distinguished in cluster I, which is the largest and made up of all the okra genotypes collected from the national gene bank, the Plant Genetic Resources Research Institute at Bunso with the exception of UCCC6. At 25% genetic dissimilarity, all the 21 okra genotypes were fully distinguished in the dendrogram. The dendrogram generated from genetic distance matrices gave an overall pattern of variations and relatedness among the okra genotypes, which agreed with the observation made by Nwangburuka et al. (2011).

Indeed, the dendrogram offered distinctive synopsis of the genetic relatedness in the okra germplasm, which is in agreement with the observation made by Aliyu and Fawole (2001) as well as Aremu et al. (2007). According to Ahiakpa et al. (2013), there is a direct relation between the eco-geographical origins of okra collections and their

Table 5. Variation in the average fruit yield and phenology among 21 okra genotypes.

Genotype	Fruit length (cm)	Fruit girth (cm)	Number of fruits	Fruit weight (g)	Yield (t ha^{-1})	Days to first flowering	Days to 50% flowering
UCCC2	8.7	2.04	5	18.9	2.49	53	74
UCCC3	8.1	2.24	4	23.6	2.57	54	68
UCCC4	8.8	2.06	4	19.3	2.23	51	70
GH2026	8.3	2.7	7	16	3.77	96	112
GH2052	8.1	2	3	14.8	1.55	105	130
GH2057	8.6	2.98	6	23.6	4.41	77	80
GH2063	8.6	2.03	2	16.1	0.86	94	125
GH3731	6.6	2.66	3	18.9	1.39	74	92
GH3734	5.8	2.59	6	17	2.85	84	85
GH3760	12.4	2.16	5	26.8	3.43	66	93
GH4374	6.6	3.03	4	15.3	1.68	104	120
GH5302	7.9	2.18	4	13.6	1.59	111	135
GH5321	7	3.11	8	21.1	4.96	52	77
GH5332	14.2	2	20	21.4	11.88	97	101
GH5786	7.3	2.67	3	16.3	1.43	102	120
GH5793	7.8	2.45	4	14.7	1.5	109	129
UCCC5	8	2.11	5	20.8	2.9	47	53
GH6105	12.2	2.11	15	22.6	9.34	61	92
GH6211	6.5	2.44	3	17.9	1.61	56	80
UCCC6	8.5	2.12	10	13.9	3.75	139	141
UCCC1	9	1.88	5	17.8	2.36	51	78
Mean	8.52	2.36	6.0	18.59	3.26	80.14	97.86
Lsd	1	0.2	3.5	3.1	2.09	10.7	7.6

Table 6. Principal component analysis of the 21 okra genotype showing the factor scores, Eigen values and percentage total variance accounted for by the first four principal component axes.

Character	PC1	PC2	PC3	PC4
Fruit/plant	0.162	-0.456	-0.031	0.523
Plant Height (cm)	-0.124	0.348	-0.039	0.804
Canopy Diameter (cm)	0.447	0.099	0.119	0.172
Leaf Length (cm)	0.466	0.113	0.071	0.095
Leaf Breadth (cm)	0.419	-0.252	-0.179	-0.073
Stem Diameter (cm)	0.427	0.117	0.253	-0.013
Petiole Length (cm)	0.427	0.217	-0.239	-0.169
Length of Internodes (cm)	-0.028	0.688	0.225	-0.076
Number of Branches	0. 012	0.225	-0.880	0.014
Eigen value	3.42	1.34	1.11	1.06
% Total variance	38.00	14.90	12.40	11.70
Cumulative % variance	38.00	52.90	65.30	77.00

clustering patterns in Ghana. Similarly, in the current work, the okra collections from the central region of Ghana clustered together and differentiated from those obtained from the PGRRI of Bunso. In addition, the germplasm collections from Togo especially GH2057, GH2052 and GH2063 being stored in PGRRI may be genetically similar for their close relatedness in cluster I. The distribution of okra genotypes might be influenced by farmer-consumer preferences, as well as okra trade and germplasm collection activities.

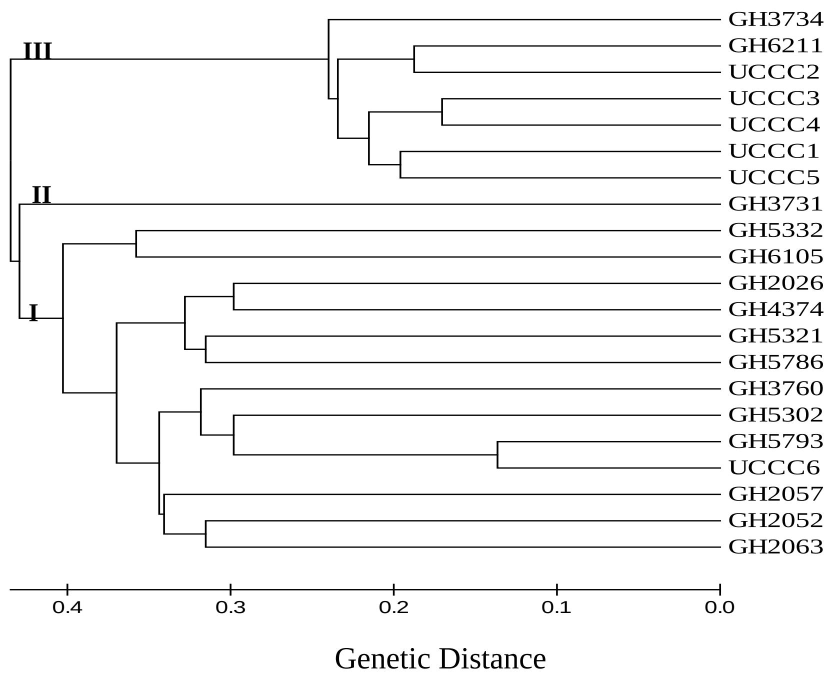

Figure 1. The UPGMA Dendrogram of genetic relationship among 21 okra genotypes based on 32 phenotypic characteristics.

Conclusions

The 31 quantitative and qualitative characters distinguished all the 21 okra genotypes without identifying clones. The discriminatory ability of the 31 characters was evident in clustering of the 21 okra genotypes in the dendrogram. UCCC1, UCCC2, UCCC3, UCCC4, UCCC5, GH6211 and GH3734 appeared more closely related. On the whole, the most diverse okra genotype was GH3731. UCCC5 had 50% flowering at 53 days which suggests very early maturity, followed by UCCC3 (68 days), UCCC2 (70 days). Though, GH5332 is late maturing with 50% flowering at 101 days, it produced a significantly ($p < 0.05$) highest fruit yield of 11.88 t/ha at a rate of 20 fruit per plant which compared well with the size of the fruits among the okra genotypes. UCCC5, UCCC3 and UCCC2 with early maturing but low yield can be hybridized with the high fruit producing, but late maturing okra genotypes of GH5332 and GH6105 to improve earliness in fruiting and adapt the crop to escape terminal drought. The most diverse genotype GH3731 could also be incorporated into breeding to broaden the genetic base of the crop. The almost 50% of the okra genotypes that produced fruits and also had large leaves suitable for use as leafy vegetables and to feed cattle could serve a dual purpose.

RECOMMENDATION

Though the phenotypic characters were useful to detect genetic variations in okra germplasm collections, they are not absolutely reliable since the traits can be influenced by the environment. Hence, there is a need to employ molecular markers to characterize the okra germplasm including exotic genotypes to establish the genetic structure of the crop and establish baseline information for breeding and conservation of the crop.

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

The authors wish to acknowledge the University of Cape Coast in Ghana for sponsorship to conduct this initial genetic diversity study in okra.

REFERENCES

AdeOluwa OO, Kehinde OB (2011). Genetic variability studies in West African okra (*Abelmoschus caillei*). Agric. Biol. J. N. Am. 2(10):1326-1335.

Ahiakpa JK, Kaledzi PD, Adi EB, Peprah S, Dapaah HK (2013). Genetic diversity, correlation and path analyses of okra (*Abelmoschus* spp. (L.) Moench) germplasm collected in Ghana. Int. J. Dev. Sust. 2(2):1396-1415.

Ali AS, Shah H, Gul R, Ahmad H, Nangyal H, Sherwani KS (2014). Morpho-Agronomic Characterization of Okra (*Abelmuscus esculentus* L.). World App. Sc. J. 31(3):336-340.

Aliyu B, Fawole I (2001). Inheritance of pubescence in crosses between *V. unguiculata* and *V. rhomboidea*. Nig. J. Genet. 15:9-14.

Aremu CO, Adebayo MA, Ariyo OJ, Adewale BB (2007). Classification of Genetic diversity and choice of parents for hybridization in cowpea *Vigna unguiculata* (L.) Walp for humid savanna ecology. Afr. J. Biotechnol. 6(20): 2333-2339.

Ariyo OJ, Akinova ME, Fatokun PE (1987). Plant character correlation and path analysis of pod yield in okra. Euphytica 36:677-686.

Ariyo OJ, Odulaja A (1991). Numerical analysis of variation among accessions of okra. (*A. esculentus* [L.] Moench). Malvaceae. Ann. Bot. 67:527-531.

Asare-Bediako E, Addo-Quaye AA, Bi-Kusi A (2014a). Comparative efficacy of phytopesticides in the management of *Podagrica* spp and mosaic disease on okra (*Abelmoschus esculentus* L.). Am. J. Expt. Agric. 4(8):879-889.

Bisht IS, Mahajan RK, Rana RS (1995). Genetic diversity in South Asian okra *(Abelmoschus esculentus)* germplasm collection. Ann. Appl. Biol. 126:239-550.

Campos ET, Espinosa MAG, Warburton ML, Monter AV (2005). Characterisation of mandarin (*Citrus* spp) using morphological and AFLP markers", Interciencia 30(11):1-14.

Chattopadhyay A, Dutta S, Chatterjee S (2011). Seed yield and quality of okra as influenced by sowing dates. Afr. J. Biotechnol. 10:5461-5467.

Choudhary UN, Khanvilkar MH, Desai SD, Prabhudesai SS, Choudhary PR (2006). Performance of different okra hybrids under North Konkan coastal zone of Maharashtra. J. Soils Crops 16:375-378.

Cudjoe AR, Kyofa-Boamah M, Nkansah GO, Braun M, Owusu S, Adams E, Monney E, Attasi R, Owusu P, Sarpong S (2005). Commercial Okra Production in Ghana - Good Agricultural Practices/Code of Practice and IPM Strategies: In: Kyofa-Boamah M, Blay E, Braun M, Kuehn A (eds). Handbook of Crop Protection. Recommendations in Ghana, Ministry of Food and Agric. Accra pp. 75-92.

Esthiet JA, Brisibe AE (2015). Morphological Characterization and Yield Traits Analysis in Some Selected Varieties of Okra (*Abelmoschus Esculentus* L. Moench). Adv. Crop. Sci. Technol. 3(5):1-5.

Hussain S, Muhammad NS, Shah A, Iqbal Z (2006). Response of okra (*Abelmoschus esculentus*) cultivars to different sowing times. J. Agric. Biol. Sci. 01:55-59.

IPGRI (1991). Okra descriptor, Diversity for Development. Int. Plant Gen. Res Inst. Rome.

Ministry of Food and Agriculture (MoFA) (2007). Agriculture in Ghana. Facts and figures. Statistics, Research and Information Division, MoFA, Accra, Ghana.

Nwangburuka CC, Kehinde OB, Ojo DK, Denton OA, Popoola AR (2011). Morphological classification of genetic diversity in cultivated okra, Abelmoschus esculentus (L) Moench using principal component analysis (PCA) and single linkage cluster analysis (SLCA). Afr. J. Biotechnol. 10(54):11165-11172.

Ogunbayo SA, Ojo DK, Guei R, Oyelakin OO, Sanni KA (2005). Phylogenetic diversity and relationships among 40 rice accessions using morphological and RAPDs techniques. Afr. J. Biotechnol. 4(11):1234-1244.

Ofoefule SI, Chukwu AN, Anayakoha A, Ebebe IM (2001). Application of Abelmoschus esculentus in solid dosage forms 1: use as binder for poorly water soluble drug. Indian J. Pharm. Sci. 63:234-238.

Omonhinmin CA, Osawaru ME (2005). Morphological characterization of two species of Abelmoschus: *Abelmoschus esculentus* and *Abelmoschus caillei*. Genet. Resour. Newsl. 144:51-55.

Owusu-Sekyere JD, Alhassan M, Nyarko BK (2011). Assessment of climate shift and crop yields in the Cape Coast area in the Central Region of Ghana. ARPN. J. Agric. Biol. Sci. 6(2):49-54.

Perdosa J (1983). The morphological characters of okra introductions. Horticult. Bras. 1(1):14-23.

Rahman K, Waseem M, Kashif MS, Jilani M, Kiran G (2012) Performance of different okra (*Abelmoschus esculentus* L.) cultivars under the agro-climatic conditions of Defra Ismail Khan. Pak. J. Sci. 64:316-319.

Saifullah M, Rabbani MG (2009). Evaluation and Characterization of Okra *(Abelmoschus esculentus* L. Moench.) Genotypes. SAARC J. Agric. 7(1):92-99.

Siesmonsma JS, Kouame C (2004). Vegetables. In: Plant Resources of Tropical Africa 2 (Grubben, G.J.H. & Denton, O.A., Eds.) PROTA Foundation, Wageningen, Netherlands and Backhuys Publishers, Leinden, Netherlands and CTA, Wageningen, Netherlands pp. 20-29.

Tamura K, Dudley J, Nei M, Kumar S (2007). MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. (Accessed on 28th March, 2016). Mol. Biol. Evol. 24:1596-1599. http://www.kumarlab.net/publications

Verma VD (1993). Collecting eggplant and okra in Madhya Pradesh and Maharashtra. IBPGR. Newsletter for Asia Pacific and Oceania 13:14-15.

Wamanda DT (2007). Inheritance studies in collected local okra (*Abelmoschus esculentus* L. Moench) cultivars. In: Combining ability analysis and heterosis on diallel cross of okra. Afr. J. Agric. Res. 5:2108-2155.

Yonas M, Garedew W, Debela A (2014). Maltivariate analysis among Okra (*Abelmoschus esculentus* L. Moench) Collections in South Western Ethiopia. J. Plant Sci. 9(2):43-50.

African Journal of Agricultural Research